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THESE
POUR LE DIPLOME D'ETAT
DE DOCTEUR EN PHARMACIE

Soutenue publiquement le 17/06/2016
Par Mme. Alice Renard

Titre

Is bacterial resistance currently driving a paradigm shift in the antibiotic industry? Novel scientific approaches, changing economic frameworks and specific regulatory measures to fix antibiotic innovation and drug development

La résistance aux antibiotiques, un phénomène actuellement responsable d'un changement de paradigme dans l'industrie antibiotique? Apport des nouvelles techniques scientifiques, des modèles économiques et des mesures réglementaires spécifiques pour relancer l'innovation et le développement des antibiotiques

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ABBREVIATIONS

ACB: Antibiotic Corporate Bond

ADMET: Absorption, Distribution, Metabolism, Elimination and Toxicity

AMR: Antimicrobial Resistance

APUA: Alliance for the Prudent Use of Antibiotics

BSAC: British Society for Antimicrobial Chemotherapy

C1G – C3G : First- / third-Generation Cephalosporins

CA-SFM : Comité de l'Antibiogramme de la Société Française de Microbiologie

CDC : Centers for Disease Control and Prevention

CDER : Center for Drug Evaluation and Research

CEESP: Commission Evaluation Economique et Santé Publique

CLSI: Clinical Laboratory Standards Institute

CMO: Chief Medical Officer

COA: Call Options for Antibiotics

CRE: Carbapenem-Resistant *Enterobacteriaceae*

DCM: Debt Capital Market

DIN: Deutsches Institut für Normung

EARS-Net: European Antimicrobial Resistance Surveillance Network

EC: European Commission

ECDC: European Centre for Disease Prevention and Control

EFSA: European Food Safety Authority

EMA: European Medicines Agency

ESBL: Extended-Spectrum β-Lactamase

EUCAST: European Committee on Antimicrobial Susceptibility Testing

FAO: Food and Agriculture Organization

FDA: Food and Drug Administration

FTICR-MS: Fourier Transform Ion Cyclotron Resonance – Mass Spectrometry

GAIN: Generating Antibiotics Incentives Now

GDP: Growth Domestic Product

GP: General Practitioner

HTS: High-Throughput Screening

HRMS: High-Resolution Mass Spectrometry

ICU: Intensive Care Unit

MDR: Multi-Drug Resistant

MIC: Minimum Inhibitory Concentration

MRSA: Methicillin-Resistant *Staphylococcus Aureus*

NICE: The National Institute for Health and Care Excellence

NGO: Non-Governmental Organisation

NME: New Molecular Entity

NRPS: Non-Ribosomal Peptide Synthetase

OECD: Organisation for Economic Co-operation and Development

OIE: Office International des Epizooties (International Office of Animal Health)

PASTEUR:

PKS: PolyKetide Synthase

R&D: Research and Development

SAR: Structure-Activity Relationship

SMEs: Small and Medium-sized Enterprises

TATFAR: TransAtlantic Task Force on Antimicrobial Resistance

VISA : Vancomycin-Intermediate *Staphylococcus Aureus*

VRE: Vancomycin-Resistant *Enterococci*

VRSA: Vancomycin-Resistant *Staphylococcus Aureus*

VSSA: Vancomycin-Susceptible *Staphylococcus Aureus*

WHA: World Health Assembly

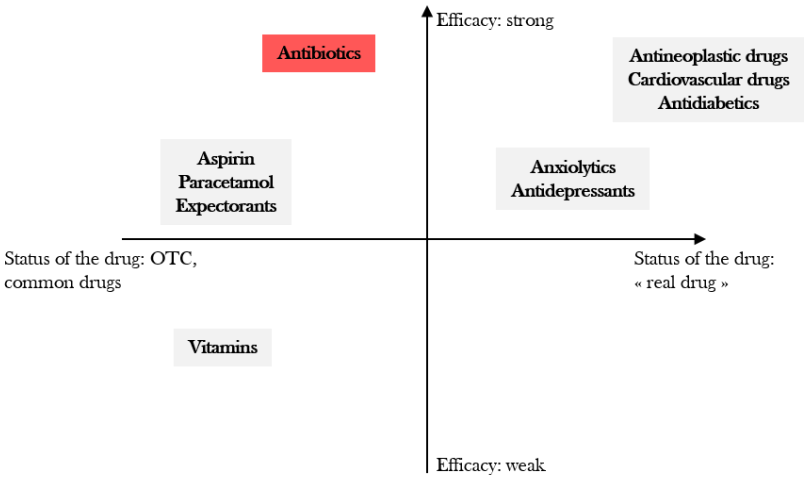
WHO: World Health Organisation

INTRODUCTION[1,2]

"What makes antibiotics unusual is that their very use undermines their future usefulness, as bacteria evolve resistance" [1]

Whereabout are we in the current struggle to contain and curb bacterial resistance to antibiotics? There is a lot of information available between the plethora of scientific articles and media sources painting the gloomy view of an antibacterial drug armamentarium eroded by resistance, and the growing public attention that the cause is getting.

In the public policy and scientific world, antibiotics are currently very much associated with resistance and the recognition of an issue that needs to be addressed. Yet such an awareness is not that commonplace among the general public. Due to their central place in therapy and their frequent use for indications spanning across the lifetime, antibiotics are anchored in a most well-known and routine universe. Should they be questioned about antibiotics, patients would willingly describe ordinary, efficacious and harmless drugs that they are so familiar with as to handle them with less care and attention than other therapeutic class (Figure 1).



[1] Antibacterial resistance, antimicrobial resistance, antibiotic resistance and resistance are used interchangeably to designate the topic of bacterial resistance to the therapeutic agents that target them. Similarly are antibiotics, antimicrobial or antibacterial drugs considered in this thesis to represent the same category of drugs.
[2] Although it is a bacterium whose resistance is a main issue and a global public health concern, *Mycobacterium tuberculosis* and tuberculosis are not considered here for its specific features that would grant them a separate discussion.

*Figure 1. The comparative image of antibiotics (survey of paients, France, 2002). From [**2**].*

Although this survey dates back to more than a decade, its results were recently confirmed by a 2015 UK qualitative survey into people's relationship with antibiotics [372]: emphasising the familiarity with the drugs was the finding that people thought they knew when they needed antibiotics and did not need the doctor to tell them; the great majority of them (70%) also felt satisfied and even more (80%) reassured when they hot prescribed antibiotics. More worringly, most did not really know what resistance was.

Hence, despite countless warnings of their dangers, the public seems to still hold on to a general belief inherited from the optimism generated by the promising, early days of antibiotics. This era has however evolved with the emergence of bacteria which display resistance to several of these acclaimed drugs. This unfortunate legacy does not only result from the complacent use bred by familiarity of the drugs, it is also a consequence of a past trial-and-error antibiotic administration, unregulated sales in the developing world, poor compliance, misuse and overuse, banalised administration in the agricultural sector, as well as the evolutionary mechanisms that enable bacteria to switch to a tenacious survival mode. Nowadays, the antimicrobial resistance problem presents an ever-increasing threat to our public health. Global in its geographic, therapeutic and bacterial scale, it involves all major pathogenic bacteria and antimicrobial drugs. Among the consequences, not only does antimicrobial resistance complicate treatment choices and puts affected patients at increased risk of adverse outcomes, but it also adds to the ever-rising healthcare costs; these outcomes, as well as the alarm bells set off one after another by scholars of authority or politicians, are what Part 1 highlights. With the additional concern of a lack of innovative antibiotics in the pipeline, the resistance issue implies that there is a need to not only double our efforts to preserve the existing antibiotics, but also to revive drug discovery routes with innovative scientific techniques and incentivise drug developers to bring new antibiotics to the market; this is what Part 2 and Part 3 respectively tackle.

Hence, the optimal management of antibiotic effectiveness, determined by the biological dynamics of bacterial evolution to resistance and, broadly speaking, the market for antibiotics, will provide a solution to a tough scientific equation with economic and regulatory parameters.

This thesis, building on theoretical concepts and objective science, strives to provide an answer to the current challenges that the antibiotic industry faces. What should be addressed, at the industry-wide and public health level, to best improve health outcomes from infectious diseases and drive innovation in this particular field? In terms of innovation, how unique and beneficial is the representant of a new chemical class of antibiotics, teixobactin? Can it currently be useful and viable? More than an analysis of the current state of the art in the antibiotic field, this thesis aims to provide a framework to develop innovative antibiotics: it holds the view that we have not exhausted

all the (scientific) options, and that, with sound economic and regulatory measures, new drugs could more easily come to market. Building on the example of teixobactin to introduce original methods for drug discovery and analyse the current market structure and actors, it develops concepts grounded in economic theory to reshape the antibiotic market.

Hence, the original contribution of this work resides in the fact that it brings together the perspective from science, with its view on antibiotic research and development, as well as a focus on market forces, with the question of regulation and economic challenges (notably through its take on incentive models). Additionally, it describes two novel, path-disrupting ideas to shift the current economic and commercial paradigm and, supported by quantitative analysis, discusses their fitness to the antibiotic industry. The macro considerations (for instance, the general economics of antibiotics, the principles of resistance) are alternated with more granular views (e.g. applications to teixobactin, the various industrial actors of the market) that are as many examples to make this work pratical and implementation-oriented.

Antibiotic resistance is a field of profuse research, even saturated in some areas. This thesis does not attempt to summarise the massive clinical and economic literature on the subject, nor is it adamant that one comprehensive and universal solution exist or be even possible. Instead, it seeks to provide a theoretical and practical framework for choosing among the regulatory, scientific and economic strategies, taking all the moving parts into account, and to contribute innovative frameworks to revamp the antibiotic industry. This thesis, with its focus on antibiotic innovation, does not address the second part of the solution to the resistance problem either, namely the conservation propositions, prescription controls and stewardship measures. Some of them are quoted to provide perspective to the question but this work does not set forth or discuss the measures to be applied to existing and already commercialised antibiotics. The focus is on what solutions should be found at the national or international level to incentivise the drug development industry back into the game.

PART 1: BACTERIA, ANTIBIOTICS AND ANTIBIOTIC RESISTANCE

As a topic that has received a lot of attention in the past few years, and whose popularity is growing in direct ratio to the media and literature exposure it is getting, antibiotic resistance is one of the main challenges that not only microbiologists, but also clinicians, public policy officers and industrials face. Yet what exactly is alarming stakeholders? What are the consequences of antibiotic resistance? This is what this initial part sets out to understand, with an analysis of the current political and epidemiological climate. It also underlines the essential features that make this problem stand out in the medical world, namely the particularities of the drugs' target: bacteria. Living organisms, they are employing cunning evolutionary strategies to circumvent the action of antibiotics: this is the core principle of resistance. Understanding its origins, mechanics, and properties is key to not only thoroughly grasp the issue of antibiotic resistance, and how it threatens treatment efficacy, but also to predict and anticipate the evolutionary game between mankind's drugs and these unicellular species.

SECTION 1. CONTEXT: ANTIBIOTICS AND ANTIBIOTIC RESISTANCE IN 2016. A "CROSS-BORDER SLOW MOVING TSUNAMI"

A. A BURNING ISSUE ON THE POLITICAL AGENDA

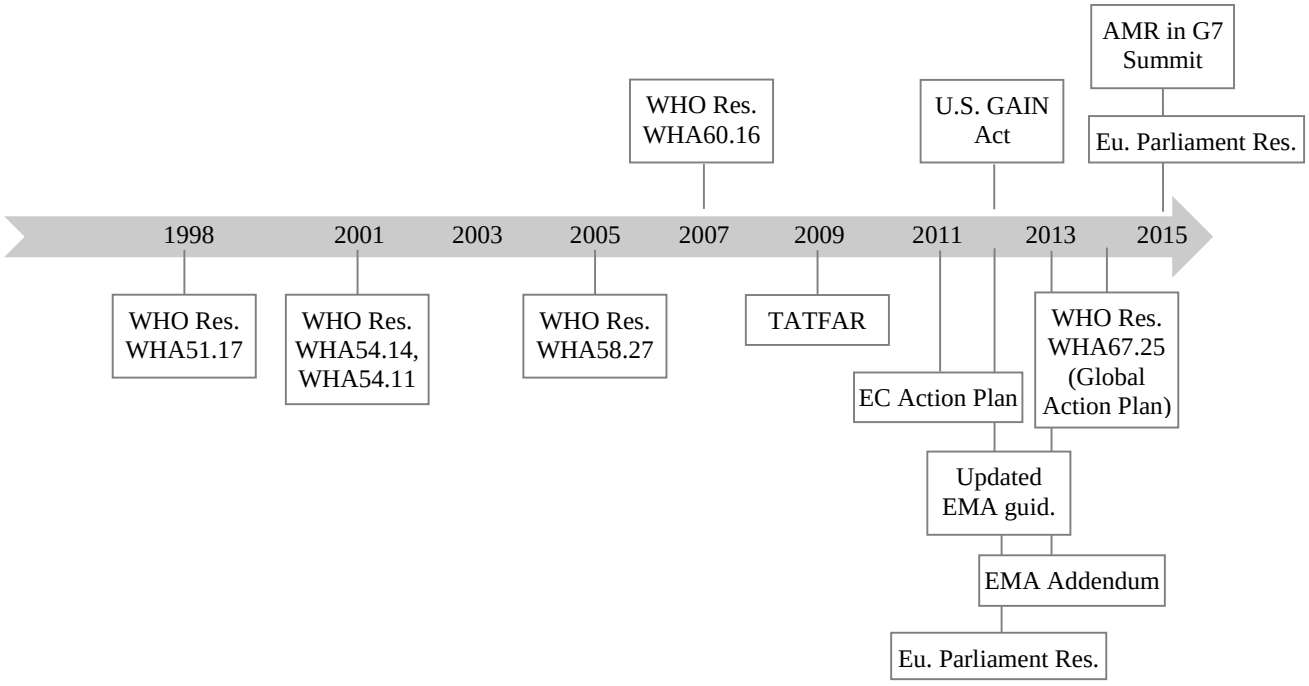
"Antimicrobial resistance is not a future threat looming on the horizon. It is here, right now" [3]; "The rise of antimicrobial resistance is a global health crisis. Medicine is losing more and more mainstay antimicrobials as pathogens develop resistance" [4]; "We face a crisis. We are hearing one alarm bell after another" [5]. Whether it is in her opening remarks at the Ministerial Conference on Antimicrobial Resistance in June 2014, in her address to the G7 Health Ministers meeting in October 2015, or at the most recent European Union Ministerial Conference on antimicrobial resistance in February 2016, Dr Margaret Chan is strongly advocating a change regarding the current situation on antimicrobial resistance, tying up the subject in her speeches to the use of antimicrobials in animal husbandry, over-the-counter and counterfeit issues in the developing world, overprescription, and solutions to stimulate necessary R&D.

Concerned about what her government recently classified as a civil emergency in its 2015 National Risk Register, alongside terrorism, climate change or the flu pandemic [6], Professor Dame Sally Davies, the UK's Chief Medical Officer (CMO), is another leading figure warning against "a ticking time bomb" that may lead to patients "go[ing] into hospital in 20 years for minor surgery and die

because of an ordinary infection that can't be treated by antibiotics" [7]. In addition to having written a volume on the problem [8] and proposed some recommendations in the CMO Annual Report 2012, she persuaded the Prime Minister, Mr Cameron, to set up an independent commission of international experts, the Review on Antimicrobial Resistance, to analyse the global problem of antimicrobial resistance and propose concrete actions to tackle it worldwide [9]. The economist Jim O'Neill, chair of the review, compared the challenge of resistance to climate change "in that it affects everyone and can only be tackled with cross-border cooperation" [10].

As Mr. O'Neill pointed out, much has been written about the topic, but in contrast to a substantially-mounting body of scientific evidence, little has been done. Antibiotic resistance had been highlighted by scientific champions as a problem in the early decades of the twentieth century, and Alexander Fleming, the discoverer of penicillin himself, had warned against this advent in his 1945 Nobel Prize acceptance speech: "The time may come when penicillin can be bought by anyone in the shops. Then there is the danger that the ignorant man may easily underdose himself and by exposing his microbes to non-lethal quantities of the drug make them resistant" [11]. But scientific issues need not only to mobilise the majority of the community or find strong champions to raise public awareness, they also need to get onto the political agenda in order to trigger the necessary regulatory moves and funding considerations. The politicisation of antibiotic resistance became the major reason for a closer attention to antibiotic use, and it is not until the late 1990s that it began to take off.

While it had been almost completely silent on the issue during the 20th century, and despite receiving advice and collaboration from the international organisation APUA (Alliance for the Prudent Use of Antibiotics, 1981) with regards to worldwide resistance surveillance efforts, the WHO started to recognise the issue in several resolutions on the subject, between 1998 and 2014 (Figure 2).



(Previous page) Figure 2. Evolution of the politicisation of antibiotic resistance through selected examples: WHO resolutions (World Health Assembly Resolutions), international networks such as TATFAR (TransAtlantic Task Force on Antimicrobial Resistance), U.S. laws: GAIN Act (Generating Antibiotics Incentives Now) and EMA (European Medicines Agency) guidance on the evaluation of antibacterial products. Res = resolution; EC = European Commission; Eu = European; Guid = guideline. Sources: [12], [13].

Taking further action in response to this growing level of concern has the international agency adopted a Global Action Plan at the World Health Assembly in May 2015, laying out strategic objectives emphasising among others the need for education, surveillance, and sustainable investment, as well as a framework prompting the member states to put national action plans in place within the next two years [14]. An action plan against the rising threats from resistance has also been communicated in 2011 from the European Commission [15] and antimicrobial resistance was on the agenda at the G7 Health Ministers summit in Berlin, in October 2015, where the Global Union for Antibiotics Research and Development (GUARD) initiative was approved [**16**]. At the national level, the United States have enacted in 2012 a law to incentivise antibiotic R&D notably with a five-year extension to an antibiotic's market exclusivity (GAIN Act) (see Part 3, Section 2, C.), while many other acts have been introduced and are currently being discussed (REinvigorating Antibiotic and Diagnostic Innovation (READI) Act of 2015; Promise for Antibiotics and Therapeutics for Health (PATH) Act of 2015; Developing an Innovative Strategy for Antimicrobial Resistant Microorganism (DISARM) Act of 2015; Helping Effective Antibiotics Last (HEAL) Act of 2015) [17].

After the global WHO, the drug regulatory agencies on both sides of the Atlantic also took their share of the involvement. Established following the EU-US summit in November 2009 was the TATFAR (TransAtlantic Task Force on Antimicrobial Resistance) initiative, a transatlantic organisation with an aim to increase communication and coordination between both regions, on topics such as the use of antibiotics in human and veterinary medicine, prevention of resistant bacteria or R&D for new antibacterial agents. While the FDA has not yet implemented significant changes with regards to the clinical requirements for evaluating and granting approval to antibiotics, its European counterpart, the EMA, which has expressed its "concern about the development of antimicrobial resistance [...] made worse by the fact that few new antimicrobials have been authorised over the past few years", has updated its guideline on the evaluation of antibacterial products in January 2012 (CPMP/EWP/558/95 rev2) and provided an additional piece of information on clinical trials for anti-infective agents according to specific indications (EMA/CHMP/351889/2013) a year later. At that same period, the agency also hosted a workshop with the European Commission to discuss legislative options to bring new antibiotics to patients and combat resistance [18]. The developing countries are not an area of focus for this thesis but it is noteworthy that a growing awareness around the burgeoning and neglected problem of resistance in these states is laying the foundations for formal recognition and action, and an example of a

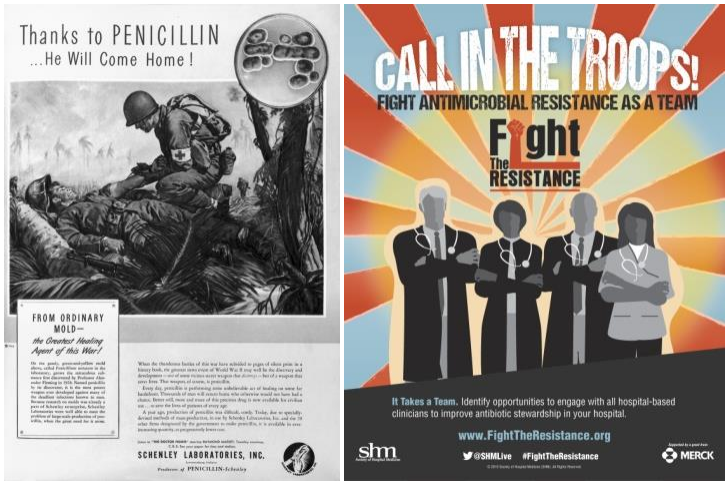
political commitment has been articulated in 2011 through the Jaipur Declaration on AMR signed by the health ministers of the 11 member states of the WHO South-East Asia region [19]. Pledging to contribute data for a regional surveillance system and to take part in a consultative process is one of the first steps for this region that could also be taken up by other developing regions, similarly characterised by a lack of systematic efforts to organise epidemiologic surveillance.

Antibiotic resistance is a topic that requires geographic cooperation from regulatory agencies with mirror expertise as well as cross-sectional competence brought by concerned authorities. The WHO recognised this need for a multi-partite engagement when it set up a collaboration with the International Office of Animal Health (OIE) and the Food and Agriculture Organization (FAO), united under the "One Health" banner [20]. Formal efforts such as the Advisory Group on Integrated Surveillance of Antimicrobial Resistance offer solid foundation for joint work on the action plans: for instance, while the OIE set standards for responsible use of antibiotics in animals, the FAO provides data on meat consumption and transmission of resistant bacteria through food. The same multi-stakeholder political awareness of the resistance problem and the need to take urgent action has been recognised at the European level, including both the European Parliament [21], [22] and the Council [23] stating their positions and acknowledging the interconnection between animal health, human health and ecosystems with the necessity for a One Health approach, and the collaboration with the European Food Safety Authority (EFSA) for its surveillance of resistance in animals and foods or additionally the European Centre for Disease Prevention and Control (ECDC) providing epidemiological updates and scientific advice on threats to human health by infections diseases. International and national policy interventions among the G7 member states have been reviewed by the OECD recently [24].

For all these organisations, and together with the media involvement with stories on the "superbugs" and the "post-antibiotic era" news, antibiotic resistance resulting from infections with limited therapeutic options is nowadays rising high on the political and societal agenda. While actions to turn the tide are beginning to be taken at the highest levels, making this threat recognisable to the general public is similarly important. There are still widespread misconceptions around the effectiveness of antibiotics, a "pill-for-every-ill" status dating back from the massive advertising campaigns of the early antibiotic days that, in 2013, still lead almost 50% of EU27 respondents to believe that antibiotics kill viruses [25]. GPs are prompt to acknowledge that the view of antibiotics as both 'miracle' and 'common' drugs is still pregnant in the public collective mind, but patient knowledge on basic issues regarding antibiotics is often quite low: for instance, some patients share the belief that it is the human body, in contrast to the bacteria, that acquires resistance to the drugs [26]. Various national campaigns and more global awareness days are developed to correct the public opinion and shifts (or even u-turns) can sometimes be highlighted in the communication

trends (Figure 3). With adverts considered as proxies for the public consideration of antibiotics and their social place, a clear paradigm shift has been initiated at the patient level.

A.



B.

Figure 3. Advertising in the early antibiotic era and in recent years. A: advert for penicillin (1944) and against resistance (2015), both relating to a military context; B: advert for Achrocidin (a combination of tetracycline, antihistamine, and analgesic, 1959) and for an antibiotic awareness campaign in the UK (2015), both in the context of a cold treatment.

B. EPIDEMIOLOGICAL TRENDS IN ANTIBIOTIC RESISTANCE

International policy plans and global efforts are currently put together to tackle antibiotic resistance. But what clearly lays behind this topic for it to alarm stakeholders? Identifying where we are now through the collection of data on bacterial resistance provides a prerequisite to the development, prioritisation, implementation and evaluation of strategies to tackle resistance.

As a brief introduction, further discussed in subsequent sections, antimicrobial resistance is based on the concept of drug obsolescence and arises when an antimicrobial therapy becomes less effective or completely ineffective at treating the microorganism that it is targeting; this is an unavoidable consequence of antibiotic usage, but the human handling of the therapeutic class (such as misuse, overuse, or poor adherence) is furthering the development of resistant strains. The

scientific bases for resistance development and spread are developed in Section 2 of this Part. Although this phenomenon has not been a particular health problem as long as novel antibacterial drugs were available to counter the rising incidence of resistant bacteria, the situation today is different, with regards to both the effectiveness of the drugs and the capacity of the industry to refresh the arsenal of antibiotics.

Antimicrobial resistance is responsible for highly prevalent diseases: it is estimated that one in five human infections in G7 countries is resistant to antibiotics [24]. Nine species, most of them highly prevalent in OECD countries, are to blame for the bulk of the international burden [27]; the CDC has also listed most pathogenic species according to their threat level (Appendix 1, Table 1). Among them, *Escherichia coli* and *Klebsiella pneumoniae* mainly cause nosocomial infections and are even more virulent in vulnerable populations (neonates, elderly patients); the first is the most frequent pathogen isolated during bacteremia episodes at all ages, while the second, another frequent coloniser of the human gut, can be responsible for severe and rapid nosocomial outbreaks, especially in intensive care units. Figure 4 compares their global resistance rates to third-generation cephalosporins (C3G): while *E. coli* shows high resistance percentages in India, China, and countries of the Persian Gulf, highly-resistant *K. pneumoniae* is mainly found in Russia or South

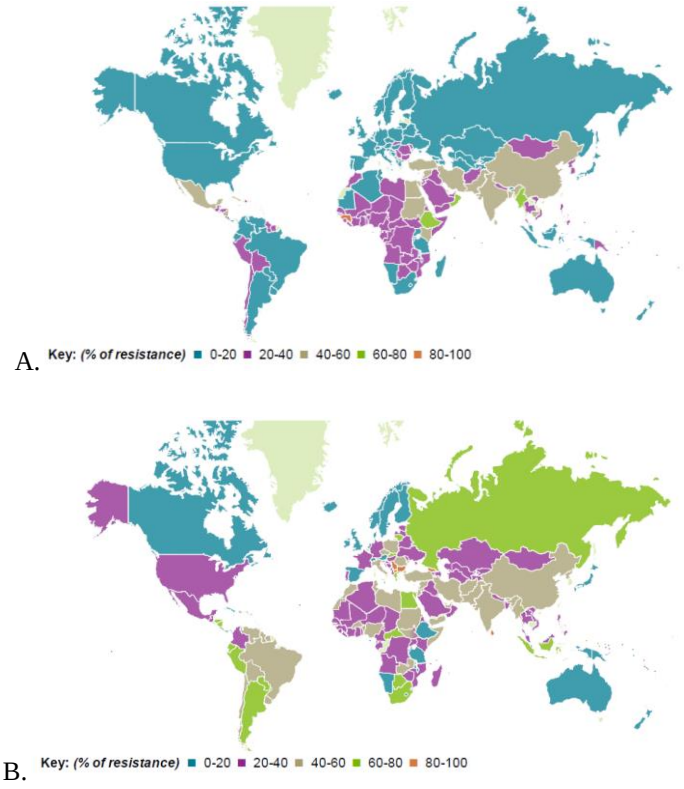


Figure 4. Global resistance rates of A: E. coli *and B:* K. pneumoniae *to third-generation cephalosporins. From [28].*

America. Following from these high C3G-resistance proportions is the consequence that treatments of these infections must rely on carbapenems, a last-resort class of drugs that in addition to being more expensive may not be available in resource-constrained systems; moreover, *K. pneumoniae* has started to show resistance to carbapenems (up to 50% in some patient groups), and of great concern is the meagre therapeutic option left for multidrug-resistant strains. Another strain responsible for nosocomial infections such as pneumoniae, commonly found in patients with cystic fibrosis, *Pseudomonas aeruginosa* is displaying resistance rates of 15-25% against leading antibiotics. To take another telling example, carbapenem-resistant *Enterobacteriaceae* (CRE), classified as one of the most urgent threat by the CDC, leave very few therapeutic options available, while their bloodstream infections have a mortality rate of nearly 50%. The rapid and global expansion of CRE is illustrated in Figure 5.

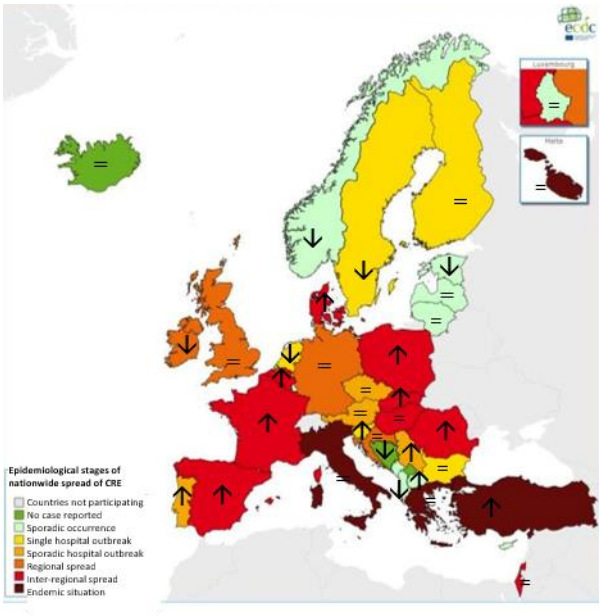


Figure 5. Situation of CRE cases in Europe and evolution. The colour blocks reflect the percentage of invasive CRE isolates in 2015. The arrows represent the 2013-2015 trends where available. From [29].

A highly mediatised bacterium, *Staphylococcus aureus*, the 'golden staph', is a very common strain in the community setting, responsible for resistant skin and soft-tissue infections. Figure 6 shows the point estimates as well as the evolution trends in the prevalence of the methicillin-resistant species, MRSA. Although European countries as a whole have seen a slight decline in MRSA infections, from 22% in 2010 to 18% in 2013, with prevalence stabilising or even decreasing in some states, the absolute figures nonetheless depict a remaining public health challenge, as seven countries reported prevalences above 25%.

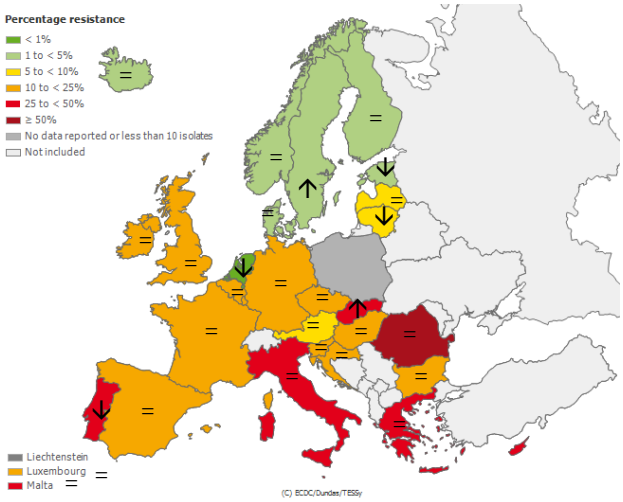


Figure 6. Situation of MRSA cases in Europe and evolution. The colour blocks reflect the percentage of invasive MRSA isolates in 2014. The arrows represent the 2012-2014 trends where available. From EARS-Net.

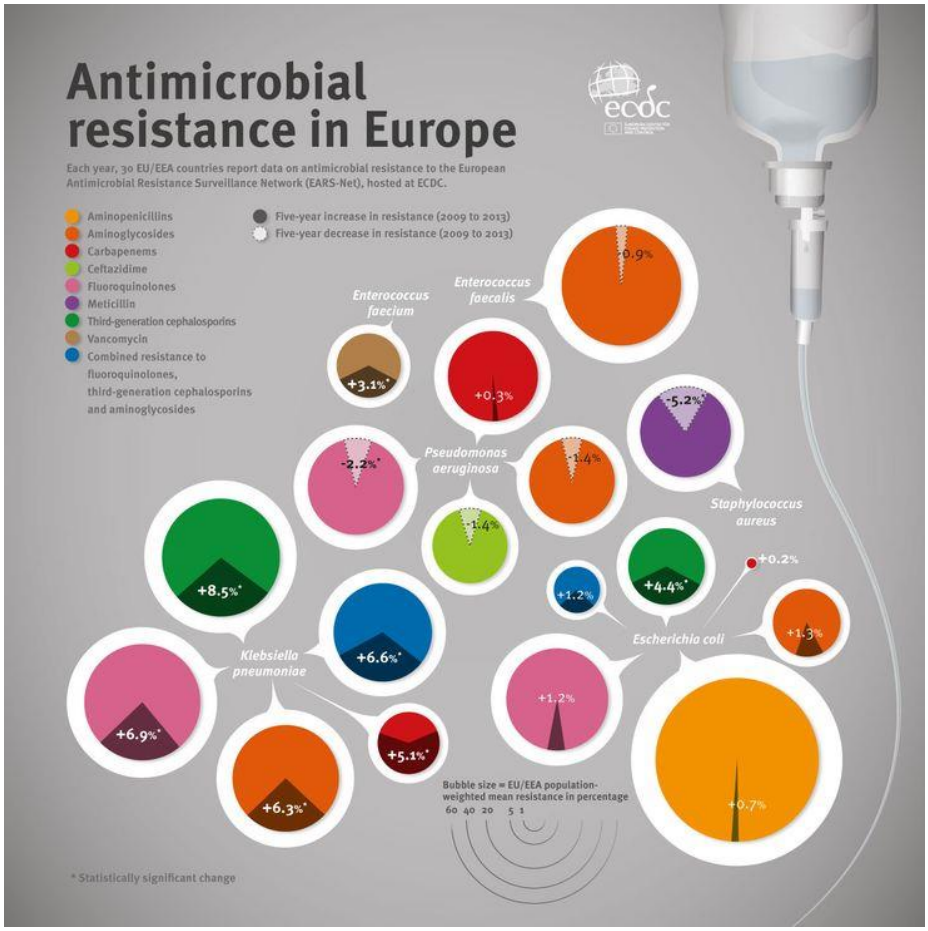
Treatment of a wound infection in such a context of high rates of MRSA will have to draw on second-line drugs such as vancomycin, while standard prophylaxis with common agents in the event of a surgical procedure is likely to be of limited effect.

Current resistance rates for Europe and the US are provided in the artworks of Figure 7. The latest surveillance reports on communicable diseases have identified emerging 2015 threats, such as the first detection of a novel resistance mechanism to colistin in *E. coli*, of concern since this antibiotic remains a last-resort treatment for infections by Gram-negative[3] pathogens. After a spread from animals to humans, a geographical spread is plausible for these strains that are currently confined to China [30]. In addition to these new threats are the evolving current ones, as bacteria in humans, food and animals continue to show resistance to many antibiotics; *Campylobacter* species continue to be highly resistant to ciprofloxacin, an antimicrobial of critical importance to treat these severe foodborne infections, while multi-drug resistant *Salmonella* strains continue to spread across Europe.

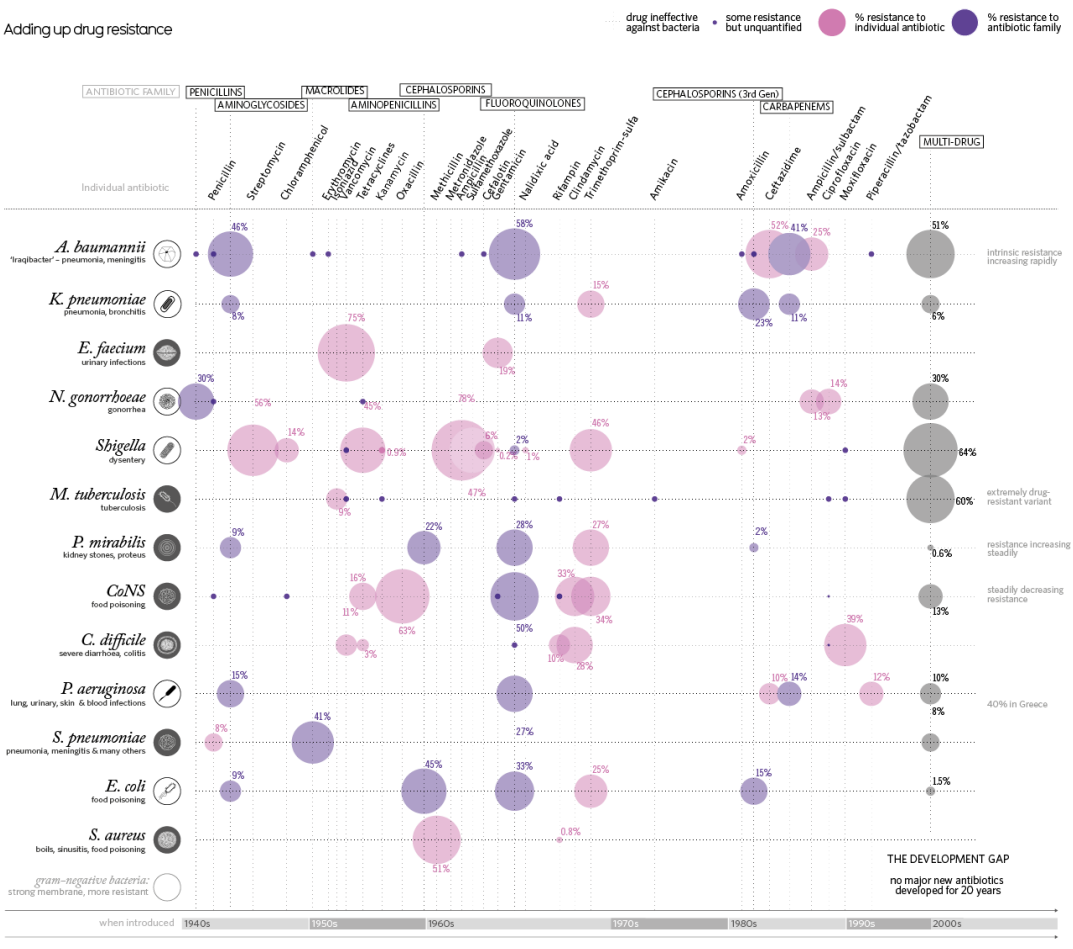
Collecting reliable information is of paramount importance to stay ahead of the race by detecting emerging problems, but also to guide policies at the national and global levels or even to assess the impact of interventions. Although resistance surveillance has been undertaken for several years in many developed countries, significant gaps, particularly in low-income regions, are an obstacle and could flaw the current picture into an underrepresented snapshot. There are discrepancies both in political will, healthcare priorities and laboratory capacities that result in various states of advancement for surveillance systems, not to mention the lack of harmonised

[3] The Gram terminology refers to the outcome of the staining used in the eponymous method to identify micro-organisms; see Section 2 of this Part.

A.


Antimicrobial resistance in Europe
ecdc
Each year, 30 EU/EEA countries report data on antimicrobial resistance to the European Antimicrobial Resistance Surveillance Network (EARS-Net), hosted at ECDC.
Aminopenicillins
Aminoglycosides
Carbapenems
Ceftazidime
Fluoroquinolones
Meticillin
Third-generation cephalosporins
Vancomycin
Combined resistance to fluoroquinolones, third-generation cephalosporins and aminoglycosides
Five-year increase in resistance (2009 to 2013)
Five-year decrease in resistance (2009 to 2013)
Enterococcus faecium
+3.1%*
Enterococcus faecalis
+0.3%
+0.9%
Pseudomonas aeruginosa
-2.2%*
-1.4%
-1.4%
-5.2%*
Staphylococcus aureus
+8.5%*
+6.6%*
+1.2%
+4.4%*
+0.2%
+1.3%
Escherichia coli
Klebsiella pneumoniae
+6.9%*
+6.3%*
+5.1%*
+1.2%
+0.7%
Bubble size = EU/EEA population-weighted mean resistance in percentage
60 40 20 5 1
* Statistically significant change

B.


Adding up drug resistance
drug ineffective against bacteria
some resistance but unquantified
% resistance to individual antibiotic
% resistance to antibiotic family
ANTIBIOTIC FAMILY
PENICILLINS
AMINOGLYCOSIDES
MACROLIDES
AMINOPENICILLINS
CEPHALOSPORINS
FLUOROQUINOLONES
CEPHALOSPORINS (3rd Gen)
CARBAPENEMS
MULTI-DRUG
Individual antibiotic
Penicillin
Streptomycin
Chloramphenicol
Erythromycin
Tetracyclines
Kanamycin
Oxacillin
Methicillin
Nalidixic acid
Rifampin
Cloramycin
Trimethoprim-sulfa
Amikacin
Amoxicillin
Ceftazidime
Ciprofloxacin
Moxifloxacin
Piperacillin/tazobactam
A. baumannii
'Iraqibacter' - pneumonia, meningitis
K. pneumoniae
pneumonia, bronchitis
E. faecium
urinary infections
N. gonorrhoeae
gonorrhea
Shigella
dysentery
M. tuberculosis
tuberculosis
P. mirabilis
kidney stones, proteus
CoNS
food poisoning
C. difficile
severe diarrhoea, colitis
P. aeruginosa
lung, urinary, skin & blood infections
S. pneumoniae
pneumonia, meningitis & many others
E. coli
food poisoning
S. aureus
boils, sinusitis, food poisoning
gram-negative bacteria:
strong membrane, more resistant
intrinsic resistance increasing rapidly
extremely drug-resistant variant
resistance increasing steadily
steadily decreasing resistance
40% in Greece
THE DEVELOPMENT GAP
no major new antibiotics developed for 20 years
when introduced
1940s
1950s
1960s
1970s
1980s
1990s
2000s

(Previous page) Figure 7. Resistance figures for selected bacteria: A: in Europe (source: EARS-Net) ; B: in the US (sources: CDC, WHO), from McCandless D. (Information Is Beautiful©).

interpretation thresholds for bacterial analyses that lead to subjective definitions of susceptibility; when it comes to interpretation, one lab's resistance is another lab's susceptibility. Indeed, the two main committees that determine the cut-off levels to categorise bacteria according to the minimum inhibitory concentrations (MIC), Europe's EUCAST and the US's CLSI institutions, have subtle but significant differences for certain antibiotic classes, and the picture is further blurred by the addition of a plethora of national committees (UK's BSAC, France's CA-SFM or Germany's DIN, to name but a few) [31]. Nonetheless, the spread of resistant bacteria has consequences that span across various areas of state concern, from threats to undermine modern medicine advances to human capital and economic losses.

C. A GLOBAL PUBLIC HEALTH THREAT

Beyond public health, antibiotic resistance is an issue of global health. The economic and political stability of any country is heavily dependent on the health of that nation; yet with the broad globalisation of the national economies, dependence is also on the health of nations of the trading partners worldwide. Emphasising this interconnectedness is the fact that antibiotic resistance spans across all countries; bacteria do not know any geographical boundaries and the detrimental health consequences from less and less treatable infections happen in both developed and developing countries, with great variations in outcomes depending on the healthcare provisions and systems, but further emphasising the need for worldwide action and coordination.

Antimicrobial resistance involves species that are responsible for many common and life-threatening diseases, acquired either in hospitals or in the community, whose treatment is becoming difficult or even impossible in some cases. Exemplifying this are common community-acquired pneumonia, which used to be readily treatable by penicillin and its derivatives, that may not respond to recommended drugs in some settings. Treatment failure can lead to reduced quality of life or ultimately to loss of life, and the burden of both morbidity and mortality has serious consequences for health outcomes. One of the proxies to measure morbidity consequent to resistant bacteria is the frequency of hospital readmissions: for example, patients infected by vancomycin-resistant *Enterococci* (VRE) have 2.7-fold increased odds to undergo a major surgical procedure and 3.5-fold increased odds to be admitted to an intensive care unit [32]. Another cause for the increase in associated poor conditions is the therapy with second-line drugs, sometimes with an adverse events profile not fully understood, or with a benefice/risk ratio higher compared to first-line drugs but tolerated because of the therapeutic option they represent. Linezolid is one of these drugs, with documented efficacy for multi-drug resistant Gram(+) bacteria; it however comes with possible

adverse reactions such as myelosuppression including thrombocytopenia and anaemia, peripheral neuropathy or lactic acidosis [33]. Resistance is also taking its toll on mortality. Patients are up to three times more likely to die [24] and the number of deaths from infections due to resistant bacteria amounts to about 0.7 million globally, and 25,000 patients each year in the EU (2011 estimates) [34]. Two-thirds of these deaths are due to Gram(-) bacteria. At the same time, bacteremia results in more deaths per year in the UK than myocardial infarction, breast, colon and lung cancer combined [35]. Highlighting significant differences in both death and hospital length of stay for resistant versus susceptible *Staphylococcus aureus* strains, Figure 8 supports the idea that resistance affects outcomes.

	Death			Length of hospital stay after surgery			
Comparison	Percentage of subjects who died	OR	*P*	Total no. of days, mean	ME	No. of days attributable to MRSA	*P*
Control vs. MRSA SSI		11.4	<.001		3.2	13.4	<.001
Uninfected control subjects (*n* = 193)	2.1			6.1			
Patients with MRSA SSI (*n* = 121)	20.7			29.1			
MSSA SSI vs. MRSA SSI		3.4	.003		1.2	2.6	.11
Patients with MSSA SSI (*n* = 165)	6.7			13.2			
Patients with MRSA SSI (*n* = 121)	20.7			29.1			

NOTE. ME, multiplicative effect; MRSA, methicillin-resistant *S. aureus;* MSSA, methicillin-susceptible *S. aureus.*

Figure 8. Health outcomes with MRSA in surgical site infections (SSIs). From [32].

An array of conditions explains this relationship: factors related to the patient, the bacterium, and the treatment may all be involved. First, the severity of the disease depends on the host's capacity to rein in the infection, and patients with compromised immunity status are likely to have poorer health outcomes, for one thing. Additionally, one could think that resistant bacteria are set apart from their sensible counterparts by an increase in virulence, although the association has not been formally confirmed [36]. A third factor, referring to treatment and care management, suggests that from a therapeutic point of view, a decreased effectiveness or increased toxicity of available drugs may contribute to adverse outcomes, as may an increased frequency of surgical interventions. Following on in that category is the delay in selecting and delivering the adequate therapy, which explains part of the increased burden on morbidity and mortality from infections caused by resistant pathogens as well; a meta-analysis identified a relative risk of delays in starting the effective treatment of 5.56 (95% confidence interval: [2.94;10.51]), which means that the therapy is more than five times more likely to be delayed for patients with resistant strains than with susceptible strains [37]. Several articles have outlined and reviewed the health consequences of infections by resistant bacteria (for instance [38], [39], [40], [41]).

Could we ultimately reach the point where the chance of developing an untreatable infection following a surgical procedure in the hospital outweighs the risks of not having the intervention in the first place? These are secondary effects that would cast a consequent shadow on the healthcare costs and the economies, which further ascertains the importance of not letting the course of resistance development evolve further down its current route.

D. A SIZEABLE ECONOMIC BURDEN

Resistant bacteria do not only do harm to the patients, they also impact the economy resulting in effects far beyond the health sector. Treating infections caused by resistant bacteria is costly when it comes to healthcare costs, not only because of the additional days in hospital that are frequently associated, but also because of the drugs required, the last-resort antibiotics or the combination therapies that are most of the time still under patent protection; taking all care factors together, the problem results in an extra spending by hospitals of $10,000 to $40,000, more than half of which is due to nursing and medical care (see Figure 9) [24].

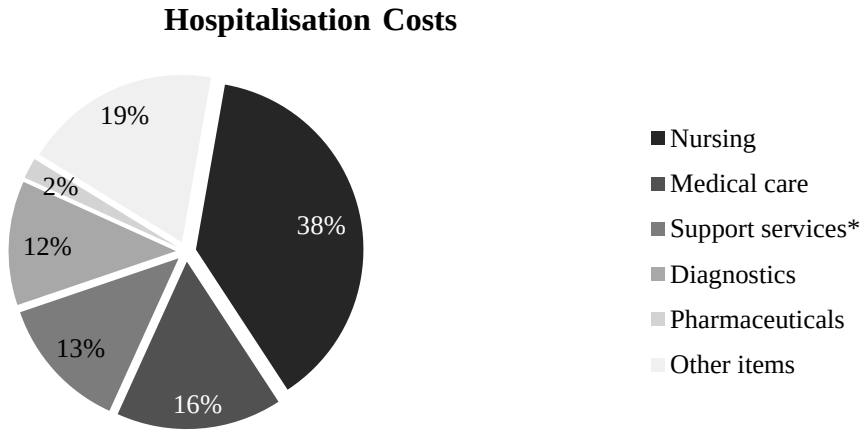


Figure 9. Costs of hospitalisation for patients with bloodstream infections caused by resistant (ESBL-producing) E. coli *strains. *Support services: e.g. laundry, food service; other items: e.g. overhead costs. Adapted from [42].*

The increased cost of therapy should not be underrepresented. While it represents only 2% in the previous figure to treat resistant *E. coli* bacteria, this figure represents a 1.6-fold increase compared to non-resistant bacteria; for another species (S. aureus) in a second study, the costs of therapy were about seven times higher [43].

Direct healthcare costs could range as high as $20 billion in the US, a figure that takes into account therapy prices, wages of healthcare professionals or even surgical interventions costs [44]. Articles and reviews of costs to the healthcare infrastructures are plentiful (see, for instance, [45], [46], [47] and [48]).

Yet because the economic burden is also to be captured through an increased level of morbidity and mortality, as previously described, resulting respectively in a labour force of lower productivity and of smaller headcount, the impact on a country's GDP encompasses societal (indirect) costs too: in addition to these two economic consequences, a third, the supply of labour, is also impacted (a caregiver might be required who would be otherwise economically productive). These societal costs are substantial: in a population of US patients, they represented more than twice the medical costs per patient [49]. At the aggregated level, extra healthcare costs and productivity losses are calculated to be at least €1.5 billion per year in Europe [34]. In the US, the societal and indirect costs, such as foregone wages, untreatable infections in livestock and economic productivity drops could total $35 billion each year. It is therefore quite clear that consequences from resistance could undermine several aspects of a country's economy. Figure 10 shows examples of the extent of economic costs, with the highest ones to be borne by low-

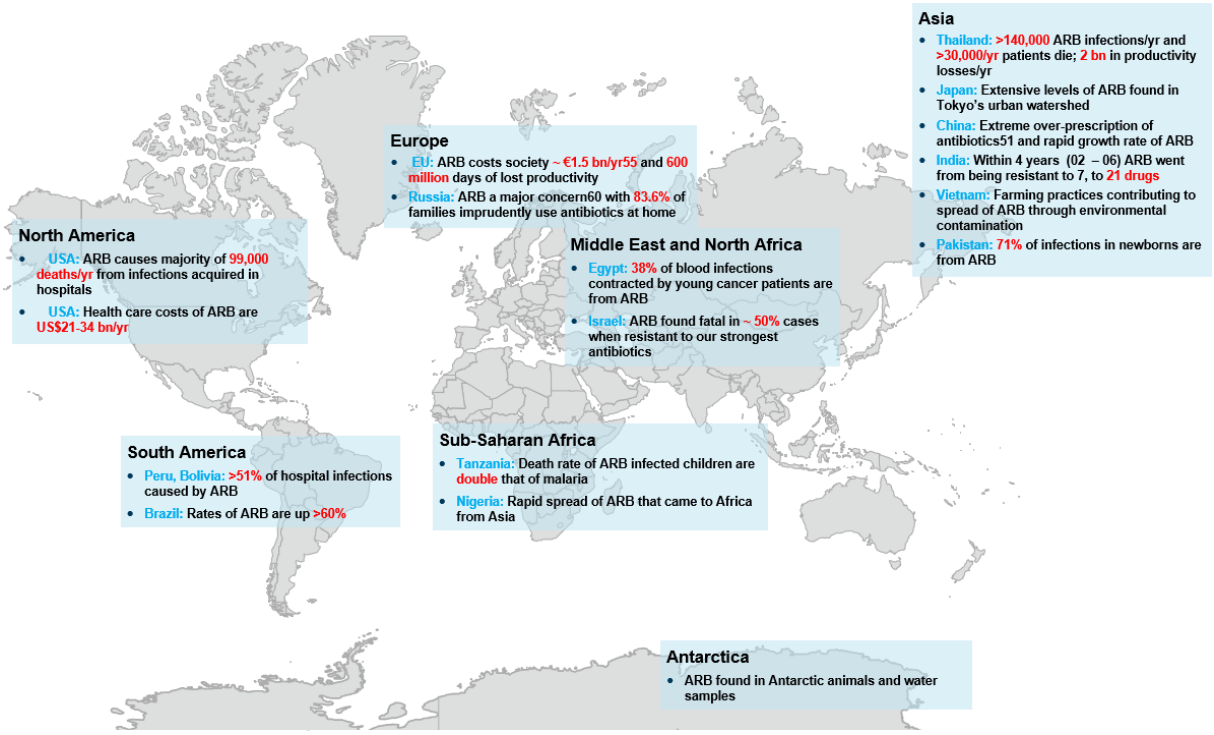


Figure 10. Global economic implications of antimicrobial resistance. ARB: Antibiotic-Resistant Bacteria. From Global Risks 2013, Eight Edition, World Economic Forum [50].

income countries, such as Africa, which could lose up to 20% of its economic output (see Appendix 1, Figure 2). This example is derived from an estimation of the future economic impact through the extrapolation of current data and trends that was undertaken in 2014 [28]. Broader results concluded to a 0.95%-5.13% reduction in GDP, depending on the country's development level, for a scenario modelling an absolute increase of current resistance figures by 40% by 2050. While this projected resistance increase is somewhat arbitrary, the study also assumed that no new antibacterial medicine

would come to market in the meantime and that the infection rates were stable (this hypothesis was varied in additional scenarii for sensitivity analyses), limitations that would suggest that the estimated impacts be more suited to illustrative rather than actionable information. To put things into perspective, another extrapolation using current rates of resistance provided by the OECD assessed the economic impact, compared to a scenario with no resistance, to reach about 0.03% of the GDP of OECD countries in 2020 and 0.16% in 2050, and this would represent a total loss of about $2.9 trillion (see Appendix 1, Figure 1) [24]. For its part, the WHO cites a global reduction in world GDP ranging from 1.4% to 1.6% [50].

Nevertheless, one of the conclusions of the first extrapolation cited above worth recalling here is that, regardless of the quantitative level of economic losses, a siloed view that would isolate them from the undeniable social loss is not a solution; and effectively dealing with antibacterial resistance will require an integrated approach, were it only for the fact that trade and agriculture are some of the sectors of the wider economy that are likely to be most impacted by the issue. There are solutions to mitigate the burden of AMR, such as preventing infections in the first place, through hygiene or immunisation, improving prescribing and promoting best practices in antibiotic stewardship; for instance, if these actions could lead to a 20% decrease in resistant infections, cost savings would range from $3.2 to $5.2 billion for the US healthcare system alone [51].

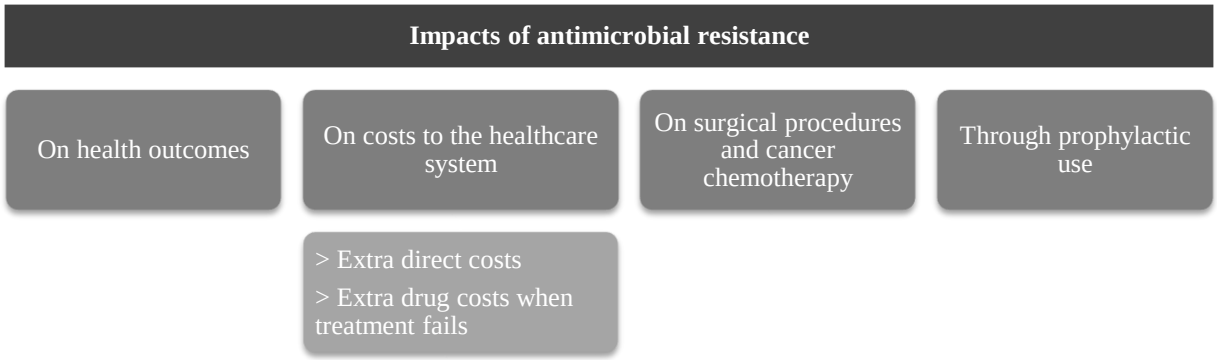


Figure 11. Impacts of bacterial resistance: conclusion. Adapted from [52].

To sum up, the emergence of pathogenic bacteria that have become resistant to several antibiotics and their diffusion in the population is one of the biggest public health threats of these last thirty years in the view of the consequences (Figure 11). This evolution is characterised by high levels of multiresistance for some species that were still classified as susceptible fifty years ago. For most of the microorganisms, exposition to an antibiotic is a *sine qua none* condition to the appearance of a new resistance mechanism and its spread among individuals. But a few bacteria develop resistance without previous exposure, by taking advantage of the mechanisms of resistant strains, as explained in the next section.

The concern of knowing where exactly does the healthcare and scientific community stand on a microbiological and technological point of view calls for a deeper understanding of both forces at play; a precise profiling of the targets – the bacteria – as well as a thorough assessment of the drugs – the antibiotics –, together with their coevolution, driven by an intertwining phenomenon specific to these two protagonists, is the topic of Section 2.

SECTION 2. BACKGROUND: BACTERIA AND ANTIBIOTICS : TWO LEADING FIGURES SHARING THE STAGE IN THE ANTIMICROBIAL RESISTANCE PLAY

Humans and bacteria have a unique relationship. An essential part of the flora of healthy individuals, the bacterial microbiome provides the environment and some of the functions for physiological activities that the host would be unable to accomplish on its own, such as the degradation of plant polysaccharides or the synthesis of vitamins B and K [53]. However, when opportunistic pathogenic species take over, the initial symbiotic relationship crumbles and a non-resolutive infection is likely to require a specific treatment aimed at destroying the responsible species with respect to the infection site in an appropriate concentration. At the macro level, antibacterial chemotherapies, epitomised by the antibiotics, have been the weapons which were believed to be as powerful as to relieve the world from the burden of infectious diseases[4]; it was without taking into account their Achilles' heel resulting from the bacteria's ability to develop resistance to these effective drugs.

A. INTRODUCING BACTERIA

a. Bacteria are ancient organisms

Bacterial life has already been flourishing on Earth for more than 3.5 billion years (possibly 3.8 billion years) by the time that ancient sedimentary structures induced by microbial metabolism were uncovered [54] [55]; although the separation from their ancestral forms, the Archaea, is likely to have occurred a bit later, both groups of living organisms constituted a common, early microbial community which the universal common ancestry was part of [56]. Not only were bacteria, as prokaryotes, the first life forms on the planet, with their emergence representing one of the most remarkable events in biological history, they have also been the dominant living group until approximately 1 billion years, together with viruses. Historically regarded as a simpler form of life

[4] William Stewart, the Surgeon General of the United States of America, said in 1967 that "The time has come to close the book on infectious diseases. We have basically wiped out infection in the United States." [83], [236].

compared to plant and animal organisms, the microbial life today represents more than half of the biomass of the planet, although the vast majority of the species have not been identified [57], [58].

Rooted in the explanation of their survival capacity and of their presence on Earth for so long is their fundamental feature of high adaptability. Rapid reproduction rates and an ability to mutate spontaneously are key for these microorganisms not only to survive in their changing environments but also to colonise different parts of the biosphere to ultimately become ubiquitous. When it comes to their first key feature, because, for one thing, of their simple unicellular organisation (lacking a nucleus, without histones associated to their DNA strands), and of their asexual reproduction mechanism consisting of independent binary division for the other, bacteria can replicate as soon as every twenty minutes in nutrient-rich conditions. For their second key feature, the ability to mutate, the genetic diversity translating into growth advantages is conferred by new mutations in the population, some of which give an advantage to mutated cells. Mutation rates are kept generally kept low by a plethora of enzymes (protection or reparation of DNA, proof-reading activities, post-replicative repair systems, for instance), but these rates can be intrinsically higher (up to 1% of a colony is made up of "mutator" clones, with constitutively high mutation rates) or due to environmental circumstances (new environment, introduction of a stress factor) [59]. Additionally, the adaptation to their environment can happen by the acquisition of new genes through horizontal gene transfer: bacteria can obtain new abilities more quickly than by *de novo* generation. These mutation phenomena are described in more detail in section 3.

Another characteristic worth noting to paint an insightful picture of bacteria's resilience is their colonisation status close to ubiquity. Bacteria have adapted to virtually every available ecological niche, even the most extreme and inhospitable ones, ranging from permafrost soils in the Antarctic to the acid flows of sulfur hot springs; they have been isolated in an unparalleled variety of habitats, not to mention the arid sands of deserts or the concentrated brines of salt lakes [60]. Thermophile or psychrophile, halophile, lithophile, anaerobic or endospore-forming species are but a few examples of bacteria thriving on very different habitats whose specific physicochemical conditions might have limited growth of any other living organisms; as a corollary, most of these species are incapable of growth on standard culture media[5]. Of example of the physiological and ecological bacterial plasticity, *Desulforudis audaxviator* is a subsurface organism which has accommodated the harsh conditions of its living environment such as a complete sunlight deprivation and isolation from other species at 2.8 kilometers underground (which got this sulfate-reducing bacterium its Latin name of "bold traveller", a reference to Jules Verne's Voyage au centre de la Terre novel) [61]. Interestingly, bacteria have also developed resistance to conditions other than naturally-occurring ones : although the rationale for such extra biological mechanisms is obscure, instead of

[5] This characteristic has implications for the identification and research of novel antibacterial compounds and is broached in Part 2.

illustrating an adaptation to environmental specificities, they might nevertheless showcase an ahead-thinking feature which could transform in a decisive edge, should the need arise. *Deinococcus radiodurans* and its record-high radiation resistance is an example of a species able to survive a dose which is 4,000 times the one that kills a human, not to say a dose that could not possibly have been emitted from natural sources on Earth [62].

All these characteristics are ingenious solutions developed to purposefully serve bacteria's ultimate goal: survival. Bacteria have outlived many other living species and are biologically well-equipped to survive evolutionary crises as well as incremental environment changes; they even have the highest capacity of all life forms to adapt to extreme and stressful ecosystems, to force evolution of otherwise invariant traits, to survive with ingenious processes.

b. Allies and Enemies

Bacteria have a very old story of their own, a predominant place in the evolutionary history of life, yet they also occupy a peculiar place in the Human life. The relationships between humans and bacteria began with the rise of the *Homo sapiens* species itself, some 195,000 years ago [63], since bacteria have always been commensal guests of humans – and have also always caused diseases. Interactions between bacteria and their hosts can take any place in the continuum of terms between symbiosis, commensalism and pathogenicity. While some species are normal colonisers of healthy individuals, they can benefit from the partnership, or confer an advantage to the other party – the host – without any harm (symbiosis), or there can be no obvious benefit, but also no detriment (commensalism). When a damage to the host is incurred, responsible bacteria range in the pathogenic category, triggering an array of immune responses to eliminate the troublesome agent.

Although they are largely ignored by the public except in the contexts of infectious diseases, the bacteria's invisible presence is essential to physiological functions, homeostasis and good health. Most impressive numbers are related to the human digestive system which harbours a record-high bacterial density of up to 10^{14} microorganisms, or an equivalent of 10^{11}-10^{12} cells by gram of colonic content; it is also remarkable that these bacteria may not only be tolerated by the host, making up a major part of their composition and living in homeostasis with their immune system, but they have also evolved to become required for their metabolic capabilities or their contribution to immunity [64]. Taking part in the gut's adaptive immunity function are healthy colonisers such as *Bifidobacterium* or *Enterococcus* species which initiate a series of reactions including transient expansion of germinal centres between B and T lymphocytes in the Peyer's patches (gut-specific lymphoid structures acting as incubators to mature these immunity cells) [65]; the skin microbiome also contributes to enhancing innate immunity in addition to maintaining a right bacterial balance: *Proprionibacterium acnes*, for example, releases free fatty acids that lower the pH of the skin surface, therefore preventing pathogens such as *Streptococcus pyogenes* to flourish [66].

Concurrently with their beneficial actions, bacteria are also responsible for a wide range of infectious diseases, whether community-acquired or nosocomial, which, differing from severity to localisations, from durations to symptoms, from targets to adverse consequences, have little in common but their causative agent, bacteria as a group (and their therapeutic response, antibiotics as a class). From the period of ancient Egypt has *Mycobacterium tuberculosis* been spreading tuberculosis, as evidenced by its bacterial DNA found in bone and soft tissue samples from mummies [67]. Bacterial epidemics have been rampant in the centuries where a deficient hygiene was only matched by the proximity of disease-carrying vectors; for instance, the bubonic plague, or Black Death, which spread in the 14th century and killed about 20 million people in Europe alone, was caused by a bacterium called *Yersinia pestis* [68]. The startling common point between some self-limiting gastroenteritis and the life-threatening typhoid fever is their identical causative species, *Salmonella enterica*, whose differences in virulence and symptoms are only explained at the serovar level, with the Typhi and non-thypoidal serovar strains. Gathered under the common term of "infectious diseases"[6], these illnesses share a similar pathokinetic pattern: the bacteria must be present in the host (colonisation) and in a multiplying stage to generate the acute infectious phenotype with clinical symptoms[7].

A vast range of conditions is caused by bacterial infections (Table 1); some can be quite severe, even fatal (meningitis), difficult to treat (osteomyelitis), benign (otitis), of varying virulence (enteritis/colitis).

Species	**Classification**	**Commonly Associated Disease(s)**
Staphylococcus aureus	Gram+	Skin and soft tissues infections; pneumonia; endocarditis; bacteremia; osteomyelitis; toxic shock syndrome
Streptococcus pneumoniae	Gram+	Otitis; pneumonia; sinusitis; bacteremia; meningitis; endocarditis
Streptococcus pyogenes	Gram+	Pharyngitis; skin infections
Enterococcus faecalis, E. faecium	Gram+	Urinary tract infections; bacteremia; endocarditis; intra-abdominal infections
Escherichia coli	Gram-	Urinary tract infections; gastroenteritis; bacteremia; skin and soft tissues infections; foodborne infections
Pseudomonas aeruginosa	Gram-	Pneumonia; bacteremia

[6] Infectious diseases are not restricted to bacteria and may also be caused by other living organisms such as viruses, parasites or fungi, but the focus here is only on bacteria.
[7] Of note, although this is the most general pattern for an infection since most of them are acute, persistent or dormant bacteria may exist in chronic infectious cases, such as tuberculosis, which, because of their specificities, are not tackled here.

Haemophilus sp	Gram-	Respiratory tract infections; meningitis; otitis; conjunctivitis
Klebsiella pneumoniae	Gram-	Bacteremia; surgical site infections; respiratory/urinary tract infections
Enterobacter	Gram-	Bacteremia; surgical site infections; respiratory/urinary tract infections

Table 1. Examples of bacteria and infectious diseases frequently associated [69].

c. Identifying bacteria

It took time (and several brilliant scientists) to actually see these "microbes", as physician Charles Sedillot designated them in 1878, before even acknowledging that they were causing many of the well-known diseases such as diarrhea, pneumonia, and numerous diseases described in terms of pain and incomfort affliciting different organs and body parts. Convincing evidence was provided from the 1860s to the 1880s by Louis Pasteur (who disproved spontaneous generation as a theory for growth of microorganisms and identified their presence in the air) and Robert Koch (who developed four basic criteria – Koch's Postulates – to link a particular organism to a disease; identified and isolated the causing agents of cholera, *V. cholerae*, and tuberculosis, *M. tuberculosis* [70]). A "golden age" for bacteriology ensued, where, in the span of 30 years (1876-1906), most bacterial pathogens causing human diseases were identified (see Appendix 2, Table 2) and with the development of the field came much of the understandings on infectious diseases, the responsible bacteria and the methods to get rid of them.

Growing and distinguishing bacteria on gelatine or agar plates has furthered the advances in aetiology and bacterial classification, with colonies identified based on their shape, size, colour – with a particularly important distinction made between two groups depending on their reaction to the Gram staining.

Gram-positive (Gram(+)) bacteria are coloured in violet after the fixing of a first stain (the crystal violet stain) to their membrane, whereas Gram-negative (Gram(-)) bacteria, whose external structures do not allow this first stain to bind, are only revealed by a second counterstain (fuchsin or safranin) that makes them appear pink-coloured. Highlighted by the duality in colouring are fundamental structural differences which, by affecting permeability to the drugs, are crucial for antimicrobial therapies. A typical, biphospholipidic cell membrane delimits the intracytoplasmic space for both categories, whereas an outer peptidoglycan (or murein) layer adds a second stratum whose dense, multi-layered network resulting from the intermolecular crosslinking of the peptidoglycan strands and associated proteins confers structural strength and form, resistance to the internal osmotic pressure and resilience to chemical and mechanical breakdown for Gram(+) species. This murein exoskeleton is also found in Gram(-) species, but in a much thinner

configuration: between one and three layers form an up-to-10 times thinner peptidoglycan, compared to Gram(+) cells [71]. To maintain an effective protection and mechanical roles despite a reduced murein wall are Gram(-) bacteria enshrouded in a third, outer layer whose lipidic and proteic content differs from the first inner membrane, notably due to the presence of lipopolysaccharides and porin proteins (Figure 12).

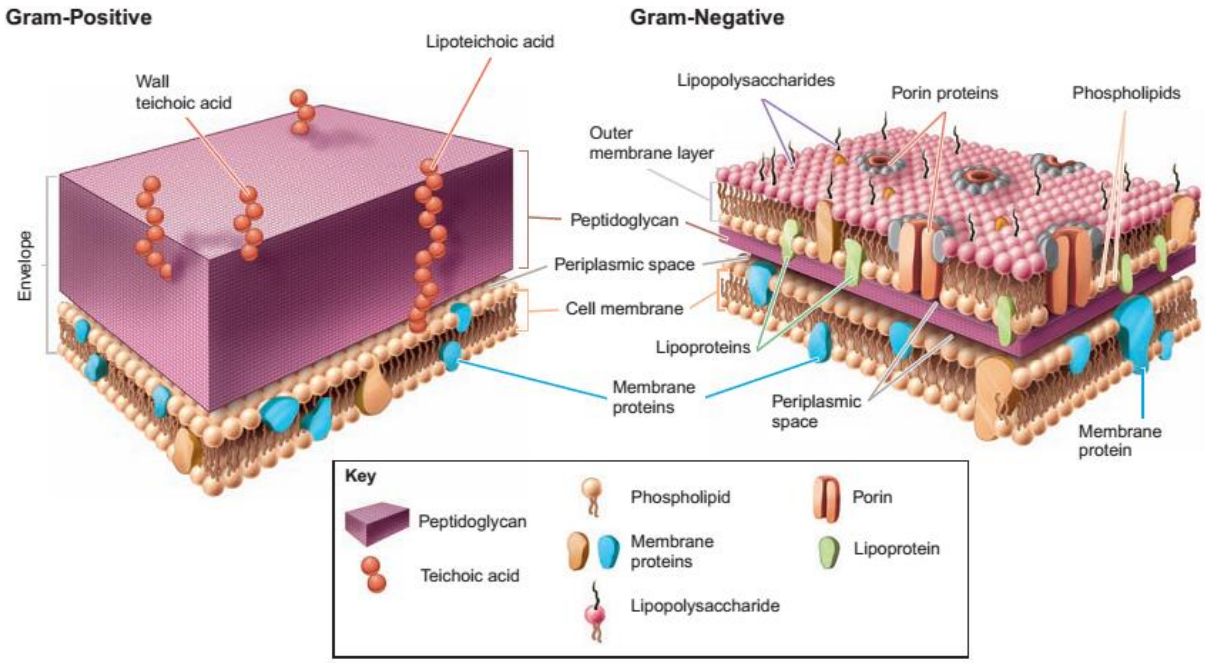


Figure 12. Structure of the Gram-positive (left) and Gram-negative (right) exoskeletons. Adapted from The McGraw-Hill Companies, Inc.

The phenotyping identification of bacteria using culture, Gram staining, and microscopy techniques have limitations that are easily overcome by molecular techniques. Among them, real-time PCR (Polymerase Chain Reaction) and microarrays are currently the most commonly employed and facilitate a rapid and accurate bacterial identification. Both techniques are based on DNA sequences; while PCR is based on DNA amplification with species-specific primers then detection of the amplicons (by hybridization with a specific probe containing a radioisotope marker, using an ELISA colorimetric method or fluorescent probe-based assays), microarray-based identification leverages pre-amplified bacterial DNA sequences to hybridise species-specific sequences, and each probe is tagged with a different coloured dye which fluoresces upon hybridization. Real-time PCR has become the most widely used molecular technology for infectious diseases diagnostics, allowing incorporation into the mainstream clinical laboratories of a rapid, sensitive and specific technique. However, this genetic technique may not be best suited to detect resistance, as only a few genes are firmly associated with phenotypic resistance (such as *mecA* or *vanA-B*) and culture remains the most clinically relevant method for antibiotic susceptibility testing [72]. Following from the growth of bacteria in the presence of various antibiotics, the antibiogram displays results

to antibiotic sensitivity tests in a qualitative scale of 'susceptible', 'intermediate' or 'resistant' categories; some of these tests can also provide quantitative results such as the MIC (Minimum Inhibitory Concentration). Such antimicrobial susceptibility testing is not only a prescriptive tool for therapy management; it can also contribute to local epidemiologic analysis when used as a periodic summary of resistance trends or as a monitoring system for resistance management plans within an institution. Finally, allowing accurate identification of any known species as well as any gene-based resistance mechanism is a most recent technique: genomics. The first complete genome to have been sequenced was that of *Haemophilus influenzae* in 1995 [73], while that of the notorious *E. coli* was sequenced in 1997. In addition to the identification power, genomics are opening a completely new field in bacteriology, whether for knowledge in essential microbiology or for drug discovery applications, since an overwhelming portion of the genes are of unknown function (this is for instance the case of 38% of *E. coli*'s 4,288 genes) [74].

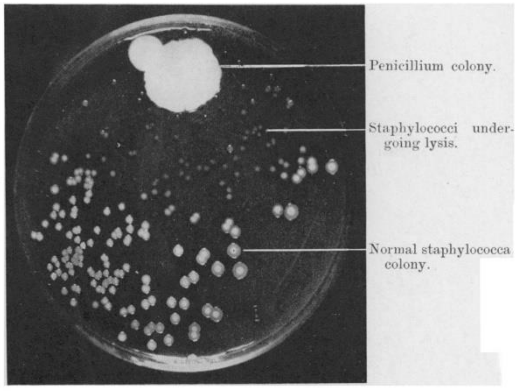
B. ANTIBIOTICS: AN ESSENTIAL THERAPEUTIC CLASS

a. Early discoveries: the beginning of the class

The methods to identify bacteria have considerably evolved, from Leeuwenhoeck's first homemade microscope in 1674 to today's genetic and fluorescence-based techniques, and with the rise in the microbiology detection capabilities came the first attempts at wiping out these unwelcome microorganisms. One early approach, defended by Koch, was to get rid of the infectious diseases through better hygiene and public health measures; another one, ardently promoted and developed by his contemporary (and often acerbically-attacked rival) Pasteur, was to protect through immunisation.

The inner, natural capability to eliminate bacteria rests on the host's defenses (mechanical, such as cilia or mucus secretions; immune, with the innate lymphocyte response or the adaptative antibodies production) which scientists have sought to aid, as their understanding of the diseases progressed, with external agents. Although the first human endeavours to chemically fight bacteria began with Pasteur's works on animal models of anthrax cured when injected with extracts of soil bacteria (1877), then Paul Ehrlich and colleagues's experiments of arsenic compounds on trypanosomes and spirochaetes (of which his first 'magic bullet', Salvarsan – arsphenamine –, came out of their search in 1908 to treat African sleeping sickness but proved active on syphilis patients, a breakthrough that not only provided the first direct treatment of the venereal disease but also opened the door to a chemotherapy era), and Domagk's Prontosil dye that cured streptococcal infections in mice (the first representant of the sulfonamide antibacterial group, in 1932), it is not before the 1940s and the penicillin that the first 'antibiotic' term was coined. The culture of microorganisms, thanks to Koch's gelatin-containing agar plate, and the postulate that substances emitted by living beings

would kill others (antibiosis) contributed greatly to the discovery and the naming of the first representant of this new therapeutic class – as did a bout of serendipity. Indeed, while returning from a particularly long and hot summer in 1928, Fleming saw a scientific opportunity in a relative hygiene slackness that had led to the contamination of his working plate of *Staphylococcus* by the fungal species *Penicillium notatum*: in the inhibited growth zone would a small compound capable of killing the bacteria be found (Figure 13). Thus was born the first commercially available antibiotic,

A. 

B. 

Figure 13. A: Photograph of a plate showing the inhibition of the growth of staphylococcal colonies in the neighbourhood of a penicillium colony (from [75]); B: Fleming in his lab, 1932 (©Science and Society Picture Library).

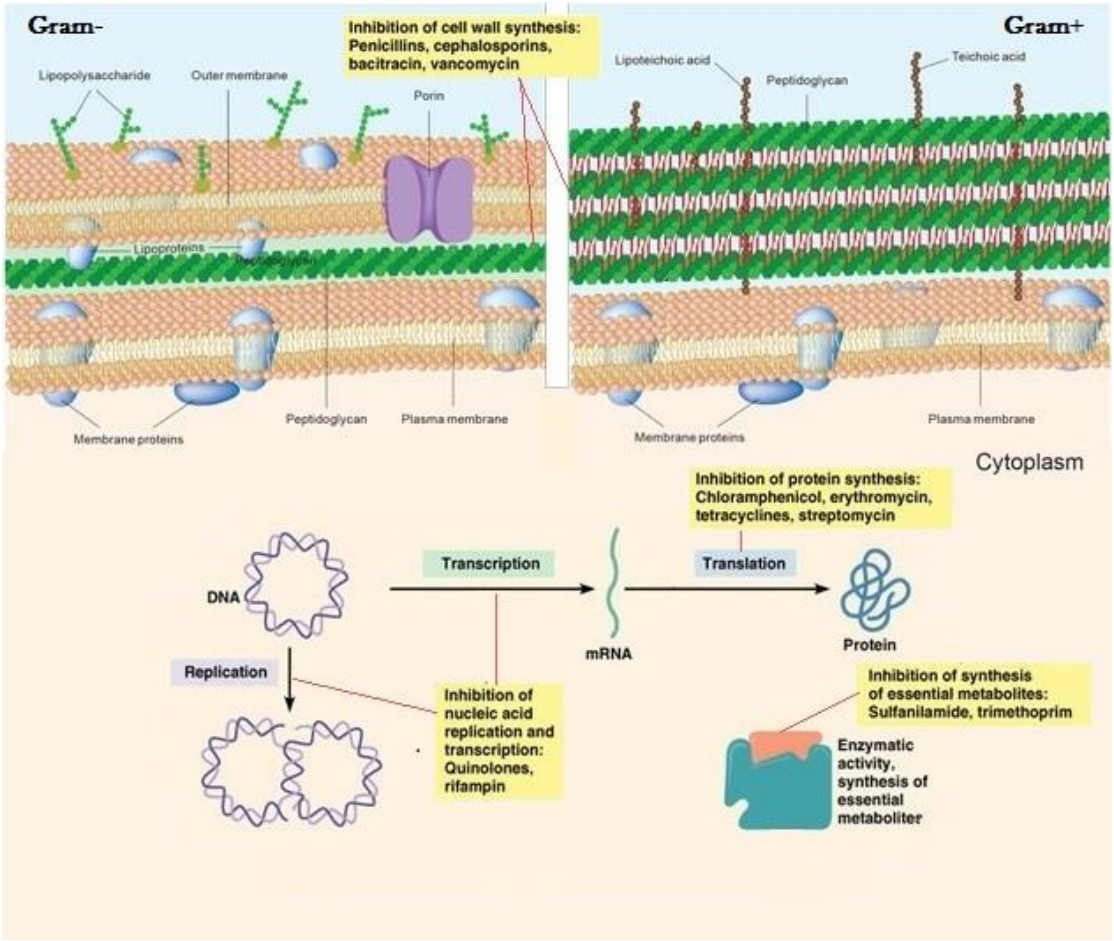
the world-renowned penicillin, a (U.S.) World War hero of its kind in the 1940s, and for the commercialisation effort must H. Florey and E. Chain be credited.

The next discovery by Selman Waksman, the American soil microbiologist, brought streptomycin and the actual term ‘antibiotic’ to gather the growing number of natural antibacterial agents. With these two antibacterial drugs, penicillin and streptomycin, began the expansion of the therapeutic class in a “golden era” that brought to market most of the drugs routinely used today.

b. Pharmacology of antibiotics

Antibiotics, agents “against life”, can either be naturally derived from producing organisms (molds) or chemically engineered by humans. What has now evolved in an industrialised therapeutic class nevertheless started with its very first representant being extracted from a natural (fungal) source, and the overwhelming majority of the drugs discovered or brought to market afterwards were of a biologic origin, whether completely natural or semi-synthetic. Antibiotics, as natural agents, are part of the never-ending competition among microorganisms for survival and dominance within an ecological niche and are by-products of the metabolism of competing species in soil, oceans or plants.

Antibiotics are small molecules that target 'microbes' (both bacteria – antibacterial – and fungi – antifungal –)[8] either in a bacteriostatic, stopping bacteria from growing, or a bactericidal, causing bacterial cell death, way. Some antibiotics can display both activities depending on the circumstances [59]. They are designed to block some crucial pathways in microbial cells selectively: exploiting the structural differences between eukaryotic (animal) and prokaryotic cells to ensure selective toxicity to bacteria only, they can either inhibit the synthesis of the cell wall, therefore disrupting the homeostasis and mechanical equilibrium of the bacterium, interfere with protein synthesis or nucleic acid metabolism, components essential to the life of the micro-organism, and other metabolic processes, for instance folic acid metabolism (Figure 14).



(Previous page) Figure 14. Antibiotic families by bacterial drug targets, with the difference in membrane structure represented for Gram(-) and Gram(+) bacteria. Adapted from Shutterstock.

The mechanisms of action of the different antibiotic families are summarised and further described in Appendix 3 (Table 3, Inserts 1-4).

[8] The focus of this thesis is on antibacterial agents only, because of the greater incidence of serious pathogenic bacterial infections, the greater number and diversity of therapeutic antibacterial drugs, and the much worrying public health threat of resistant bacteria.

c. Antibiotics, pillars of modern medicine

Emphasised above has been the case that bacteria are causing a plethora of infectious diseases, from epidemic conditions such as the plague, leprosy or diphtheria, to more common, routinely diagnosed diseases, such as otitis or urinary tract infections. Before the discovery of antibiotics, in the early 20th Century, infectious diseases were the leading causes of mortality in industrialised countries, mainly due to the poor health status of the working class [74]. Improvements in hygiene and sanitation, antibacterial therapies and vaccines where available have all contributed to a strong decline of this infectious scourge. The chemical fight against bacteria with antibiotics to restore good health from the infection really took off with penicillin: since its introduction in the clinic, penicillin is estimated to have saved the lives of between 50-100 million people [76]. Infectious diseases are still taking a considerable toll nowadays (Figure 15), with an estimate of the burden of bacterial infections alone of about 9 million deaths in 20049 [77]. However, checking on global public health advances during the first decade of the century, the CDC underlined that activities to combat major infectious causes of deaths had borne

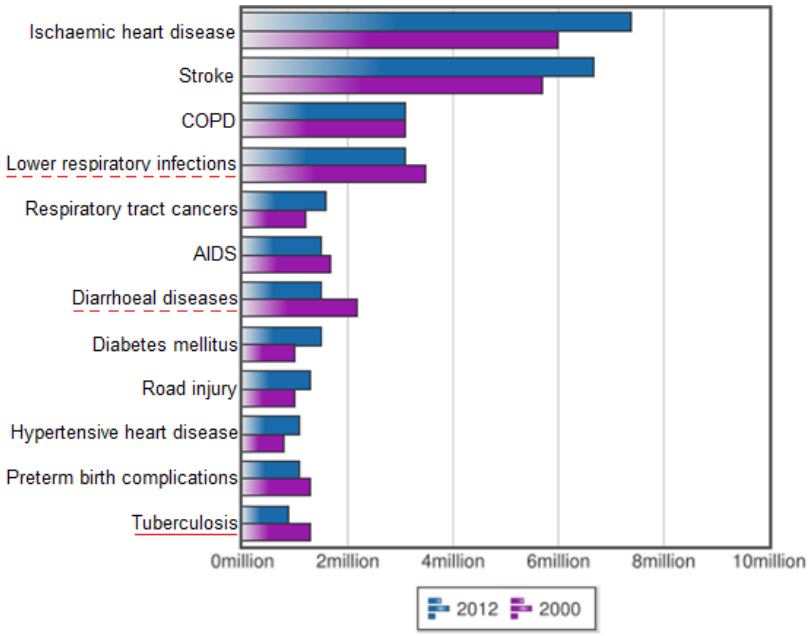


Figure 15. Comparison of leading causes of death between 2000 (violet) and 2012 (blue); infectious diseases are represented by tuberculosis and partly by lower respiratory infections and diarrhoeal diseases. COPD: Chronic Obstructive Pulmonary Disease; AIDS: Acquired Immune Deficiency Syndrome. From [78].

fruit, and while the bulk of them was viral or protozoan diseases (malaria, HIV, Guinea worm), substantially fewer deaths were also noted in bacterial diseases such as tuberculosis, diphtheria or

[9] Number worked out by adding up diseases of bacterial cause when granularity was provided (e.g. tuberculosis, pertussis) and diseases whose main causes are bacterial (e.g. meningitis). Number is likely to be an overestimation of the real figure.

tetanus. The report also identified a shift in the major causes of death from infectious to non-communicable diseases: by 2030, the latter, which include diabetes, cancer or cardiovascular diseases, are expected to be the cause for more than 75% of global deaths, regardless of the state's income [79]. There is, nevertheless, a continual evolution of a wide range of emerging and re-emerging infectious diseases, driven by technological and societal changes, similar in motion to a "restless tide" [80]: in this area, resistant (or multi-drug resistant, MDR) bacteria are examples of emerging infections, such as with vancomycin-resistant *Staphylococcus aureus*. Strategies to contain antimicrobial-resistant infectious diseases, rather than infectious diseases, may set the agenda.

Following from these evolutions are antibiotics sometimes labelled as pillars of modern medicine, and such an impression can be a consequence of their curative capacity, where many other classes of drugs only achieve a relief of symptoms. The successful treatment of the acute condition caused by a bacterium relies on several conditions, such as the identification and eradication of the bacterial strain with an antibiotic started soon enough, the absence of negative immune consequences at the host's initiative (cardiologic, arthritic...) and the absence of any definitive lesions (such as paralysis from damage in the bone marrow). If all these conditions are gathered, provided the right antibiotic is prescribed, the patient can fully recover from the infection.

Yet antibiotics are not only used for their curative powers to infectious diseases; they are a prerequisite and essential element to many surgeries and other therapeutic procedures. Modern medicine as we know it has been made possible on some accounts thanks to antibacterial prophylaxis, an invaluable therapeutic tool to undertake sophisticated interventions such as organ transplantation, cancer chemotherapy or joint replacements. Improvements in medical technology have increased the need for, and use of, antibiotics [81]: for instance, the increase in ICU patients due to a better intensive care management has led to growing the number of persons likely to develop infections (susceptible, fragile patients) and the possibilities and fertile grounds for resistant bacteria to emerge, hence growing the use of antibiotic prophylaxis. This heavy reliance on antibiotics across other applications of medicine has an importance emphasised by some authors, describing it as "the secondary health effects of antimicrobial resistance" [82]. Although quantifying its impact is difficult, as very little is known precisely about the increased odds of infections in the absence of prophylaxis or the incidence of people opting out on surgery, high-volume medical procedures that are routine but dependent upon antibiotic prophylaxis, such as caesarean sections, will definitely be impacted by a rise in bacterial resistance and in conjunction increase the burden on health outcomes.

Many factors, some just skimmed over here (but described in more detail in [83]) as well as future circumstances are conducive to the spread of infectious diseases, and the global political agenda, as

underlined in Section 1, seems to have factored in the multi-level health consequences of resistance to reflect the growing attention that the issue is receiving. The pharmaceutical industry should do as well, since the long-term profitability of their other therapies is also compromised by the rise of antimicrobial resistance.

d. Consequences of antibiotic misuse

Misusing an antibiotic is nothing like misusing a drug from any other therapeutic class, be it antidiabetic or antipsychotics for instance. Because of the characteristics of the organisms that they target (bacteria) and the nature of the diseases that they treat (infectious diseases), antibiotics can lead to a lot more adverse consequences than just inefficacy and therapeutic failure when misprescribed or misdosed.

First, antibiotics are no harmless agents: their side effects, which can be quite substantial during a normal course of therapy, can have their incidence increased to serious and fatal reactions. One well-known example is the adverse events profile of aminoglycosides, with common ototoxicities and the development of nephrotoxicity, a major limitation to their clinical use that can take the worst forms of acute tubular necrosis [84]. In an area where empiric treatment is still very common, antibiotic misuse can obscure the correct diagnosis and, at a bigger scale, deteriorate diagnostic and therapeutic knowledge, with a weakening of prescription rules and professional attitude. Antibiotic misuse does not only result in an increased incidence of bacterial resistance: an unnecessary sensitisation to the drug prevents its future use, whether for the causative bacteria at hand or a future pathogenic one. Also on the microbial side, in a patient where the normal flora has been wiped out, bacterial or fungal additional infections can occur more likely, increasing morbidity and possibly mortality [85].

To sum up, antibiotics as a class have gone through a vast amount of change paralleling not only the incremental scientific discoveries to bring new antimicrobial molecules to market but also the broader, contextual evolution of drug stewardship and regulation. Yet while the history of antimicrobials is a fascinating subject with several therapeutic and social implications[10], there is another side to the triumphant portrait of antibiotics as ‘wonder drugs’ and other ‘miracle’ pills. This aspect has been known from the very beginning, warned against by the discoverer of penicillin itself, underplayed by both most of the scientific community and the drug industry, and in the few cases when broad enthusiasm about the drugs was not on the cards, other concerns (such as safety) would prevail over this muted subject of bacterial resistance to antibiotics.

[10] This topic has been reviewed at length in S. Levy’s seminal book [341], S. Podolsky’s thorough and well-documented book [236] as well as in other reviews (see, for instance, Michel-Briand’s historical perspectives on some antibiotics [74] and Bud’s book on penicillin and derivatives [340]).

SECTION 3. A CASE OF NATURAL CO-EVOLUTION: THE INEVITABILITY OF RESISTANCE, OR HOW BACTERIA HAVE MANAGED TO ESCAPE ALL AVAILABLE ANTIBIOTICS SO FAR

Resistance to antibiotics is not a disease. It has often been metaphorised as a fight. It is only in the early 20th century that this fight took the dimension of a more organised counteraction employing chemical weapons which would give birth to a novel therapeutic class of drugs to be reckoned with: the antibiotics. First put on the track of antibacterial compounds by a fortuitous discovery, scientists have then screened, engineered and developed hundreds of compounds to manage to keep ahead of the pathogenic bacteria's outbreaks. Since then, the combat had been escalating, with temporary advantages being conceaded in turn to one side – humans – or the other – bacteria –, but nowadays the awe resulting from the discovery of such novel "miracle drugs" and the industry's frenzy for first-in-class or even me-too antibacterial drugs have slowed to a current point where our party is wondering wat its next move will be.

Why is there such a fight at all? Why do antibiotics need to evolve constantly? This is chiefly due to the unavoidability of resistance. Drawing from the above highlights on the very nature of bacteria, the inference on the unavoidability or universalism of resistance comes logically. Bacteria's only goal in life is to survive, or, in other words, to live and multiply, and each and every component of their unicellular confection is employed with this ultimate aim in mind. Responding to aggressions and adapting to changes in their environment (which antimicrobial chemotherapies are part of) had been the means of achieving such a goal of theirs for the past billion years, and so will they be for the times to come. Another way to express such an observation inferred from the prokaryotes' adaptative life strategies is that, no matter how innovative, synthetic, broad or narrow spectrum an antibiotic can be, bacteria will always and eventually have a response to these aggressive agents brought forward to eliminate them.

How has this particular, human-versus-bacteria struggle evolved? The entanglement between the drug – the antibiotic – and their target – the bacteria – is such that the coevolution is permanent, with a status-quo resulting from the stopping of any of the actors meaning the victory of the other one. Bacteria occasionally cause diseases, humans have developed drugs against them, bacteria have then created resistance mechanisms to live on and humans are consequently producing other drugs, and the story repeats in circle. On one half, the bacterial resistance techniques, no matter how widespread or specific, enable the microorganisms to escape the action of most, if not all, of the antimicrobial agents currently available, drawing on their millennia-old ability to survive. On the

other half, human creativity in drug development might meet its limits earlier than their opponent's ingeniousity's, with a global therapeutic supply of truly innovative agents running out of steam. This section describes the scientific bases that rule a battlefield unique to the antibiotic class of drugs.

A. How does resistance occur and operate?

a. How does resistance occur? The power of mutations

It has been only for the past 70 years or so that humans started to declare war on a >3.5-billion-year-old enemy: no wonder that bacteria would come equipped with numerous tools that are dedicated, or could be re-engineered, to evade the lethal action of antibiotics. But in the face of masters of the evolutionary game outpacing their hosts in experience, humans have provided witty tools and developed cunning solutions to challenge bacteria, then bacterial resistance.

Resistance is really a consequence proper to antibiotics. Broadly speaking, resistance describes the capacity of a microorganism to grow despite the presence of an antibiotic. Technically,it is characterised by an MIC for the given drug, the concentration that blocks the growth of the bacteria isolated from a patient's sample, that is superior to the value decided by experts of the antimicrobial community (CLSI in the US, EUCAST in Europe). It is important to distinguish between resistance and inefficacy of the antibiotic: while resistance is one of the explanations for a lack of efficacy of the drug, it is not the only one; conversely, it is not because an antibiotic is not effective that the bacterium it targets is necessary resistant. An example to illustrate this fact is the case of dormant strains: also called persistent, or non-multiplicative, these bacteria display tolerance, rather than resistance, to the drug, and their status of metabolic inactivity prevents them from being eradicated by the therapy.

Yet how exactly does resistance occur? By what sleight of hand do microorganisms suddenly manage to survive a compound that once destroyed them? Their resistance phenotype would need one or several resistance proteins, which are themselves derived from genes. The question then becomes: how do these resistance genes appear in the first place?

The first case is if the bacterium is itself an antibiotic producer. Then, it will have to protect itself from the lethal effect of its compound, and encoded in its genetic material will be the defense mechanisms that counter the action of the antibiotic compound, or, in other words, a cluster of resistance genes; this case is coined 'intrinsic resistance'.
If, on another hand, a bacterium is in contact with an antibiotic substance that it has to fight (hence does not possess the resistance genes yet), an evolutionary mechanism is at play. To understand the

mechanism of this second, 'acquired resistance' case is a recall of some bacterial characteristics useful. These microorganisms are very resilient, have very high replication rates and have, surely as a consequence of such intrinsic flexibility, survived for billions of years (see previous section). Because a thorough DNA proof-reading would take its toll on the replication speed and would not be compatible with a new generation every half hour or so, bacteria are often subject to DNA replication errors. The uncorrected change in the chromosomal sequence will then result in a genetic mutation: the genetic materials between the parent and child cell would differ. It is believed that this relatively high possibility of error suits the bacterial world well, otherwise evolution rules would have fixed it. Whatever the underlying factors, it has been estimated that the error rate for the bacterial DNA polymerase can be around 10^{-5} for most species, with the proofreading machinery reducing it to $10^{-7} – 10^{-6}$ [86]. In a population of 10^{11} bacteria, this means that at least 1,000 mutants are likely to appear. Considering a random distribution of mutations on the 3,000 genes or so, this means that about one-third of the genes are mutated[11]. For instance, after 20,000 generations, 45 mutations have been identified in an *E. coli* strain: 29 single-nucleotide polymorphisms (SNPs) and 16 deletions/insertions/other polymorphisms [87]. This naturally-occurring mutation phenomenon might be rare, it can nevertheless become perceivable (and clinically relevant) because of bacteria's impressive vitality, and one mutant can quickly transform into a mutated colony.

Yet a mutation does not immediately translate into resistance. The mutation can be in a non-essential or non-coding DNA zone, or, even in a coding zone, have no phenotypic consequences (expression of an unchanged protein or a modified one that keeps its original function). It is only when the resistance is expressive (with a phenotypic impact) and confers a survival advantage to the bacterium that it will result in observable resistance. Again in the *E. coli* mutations example, out of the 29 SNPs, 22 were found in coding regions, with a predominance of beneficial mutations over complete random mutations. Also, mutations are not caused by the antibiotic itself, although the drug can produce hypermutative conditions depending on its mechanism of action (for instance by generating stress conditions that activate the SOS response), but, rather, the presence of the drug creates a special environment that selects and favours the growth of the appropriate mutant strain that can thrive under these conditions. Figure 16 illustrates the interaction between mutated strains and antibiotic pressure: the colony may have a few mutated pre-existant strains (in green and violet

[11] Two phenomena can increase the mutation frequency: hypermutative strains, for instance in some *E. coli*, with a variation in their DNA proofreading machinery that increases the error rate by as much as a 10^3 factor; or mutator mutations, mutations that affect the DNA replication or proofreading tools, increasing the basal error rate too [**342**].

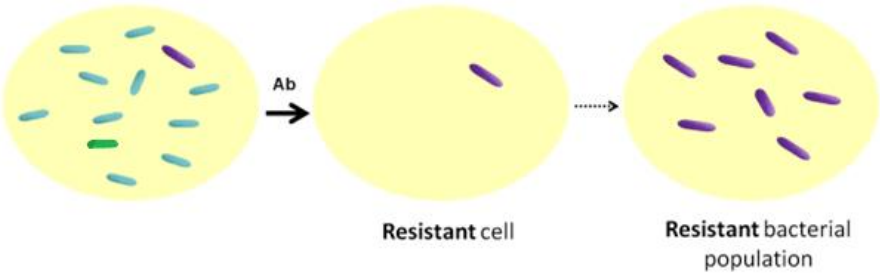


Figure 16. Apparition (and selection) of a mutated strain. Ab: antibiotic.The bacterium with different colours (violet and green) are mutated ones.

in the picture), but only the mutations that confer a selective advantage and enable their carrier to survive despite the antibiotic will be selected.

b. How does resistance spread? The concept of bacteria's "floating genome"

The newly-created resistance gene (or set of genes) can then naturally be passed on to the next bacterial generation (vertical transmission), but microorganisms have found another very efficient and rapid way to acquire a variety of genes, without having to endure the lengthy and hazardous process of mutation.

Traits chromosomally encoded are sometimes not enough for bacteria which have acquired supplementary genetic information accessible through horizontal transmission (see Figure 17). Bacteria can literally pick any gene from any other species (since the phylogenetic barriers are

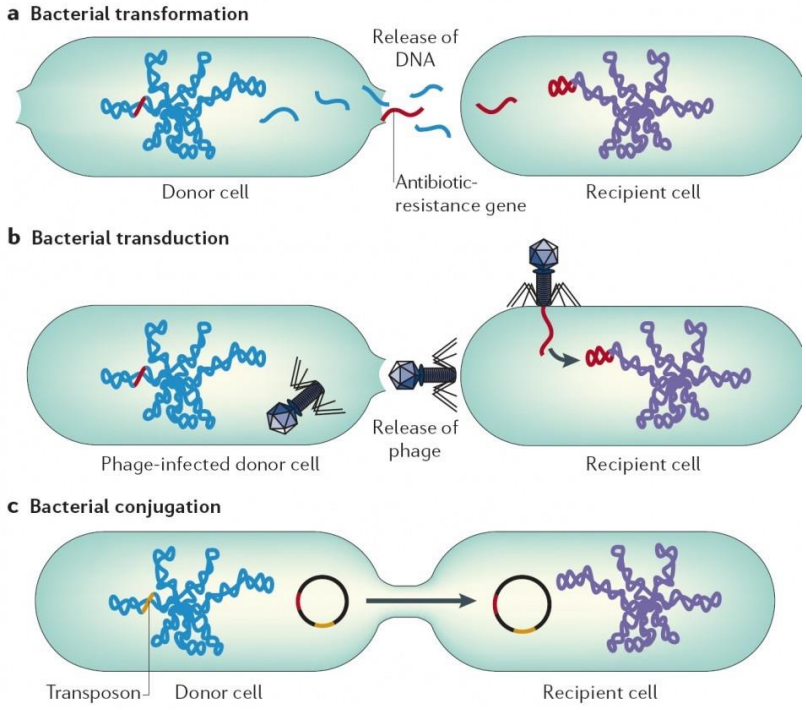


Figure 17. Horizontal gene transmission: three mechanisms. From [88].

almost non-existent), either an additional piece separated from the chromosome or incorporated into it; these are genes that are either released into the environment upon lysis of a bacterium (transformation), vectorised by a special form of virus, a bacteriophage (transduction) or transferred through direct contact between two cells, where a genetic piece independent from the chromosome of the donor cell, a plasmid, is passed on to the recipient cell (conjugation). This last mechanism explains the rapidity with which resistance can appear when two strains, a resistant and a susceptible one, are located close to each other, for example both colonisers of a patient's gut.

Plasmids and transposons, these readily available genetic materials that increase resistance and viability of bacterial species, have been gathered under the expression "the floating genome", whose importance for bacteria is undeniable. For instance, although these plasmids (also named "resistance (R) factors") have been identified since the 1960s [89], occurrences of their global spreading capacity – and thus their crucial importance in resistance – were not witnessed or given much consideration until they translated in major public health concerns.

The rapid dissemination of the New Delhi metallo-β-lactamase 1 (NDM-1) is one of the most illustrative example and a case in point. This enzyme was first identified in December 2009 in a pan-resistant strain of *Klebsiella pneumoniae*, acquired in India and carried by the bla_{NDM-1} gene [90]. But shortly afterwards (in May 2010) was the same case of resistance pattern discovered in a different species, *E. coli*, in the UK, hence quite far away from the supposed geographical birthplace [91]: the patient had visited India 18 months previously. A month later, three additional cases, with all patients having received medical care in India, were reported in the US, and later on, the gene was found in yet another species, *Acinetobacter baumannii* [92]. By August 2010, in eight month's time, there were a few cases in Canada and the US, 37 cases in the UK, 44 in Chennai and 73 in various other sites, especially in India and Pakistan (Figure 18). What mechanism other than a mobile gene-containing DNA loop, or plasmid, could explain such an ability to spread not only among species, but also across continents, and moreover so rapidly?

This conjugative plasmid was among the first ones to draw attention on the no-limit spread of redoubtable resistance, as it can harbour a high number of resistance genes in addition to bla_{NDM-1}. Indeed, resistance genes can spread between plasmids or from chromosomes to plasmids thanks to transposons (mobile DNA elements capable of moving to plasmidic structures) and integrons (sequences that gather genes encoding resistance next to an active promoter, critical for the expression of resistance genes). Examples of other horizontal resistance genes carried by plasmids are currently plentiful: highlighted by Dr M. Chan in February 2016 is the *mcr-1* gene, a novel and worrisome resistance gene reported in at least 17 countries since its identification in November 2015, which confers bacteria the ability to escape colistin, a last-resort antibiotic for

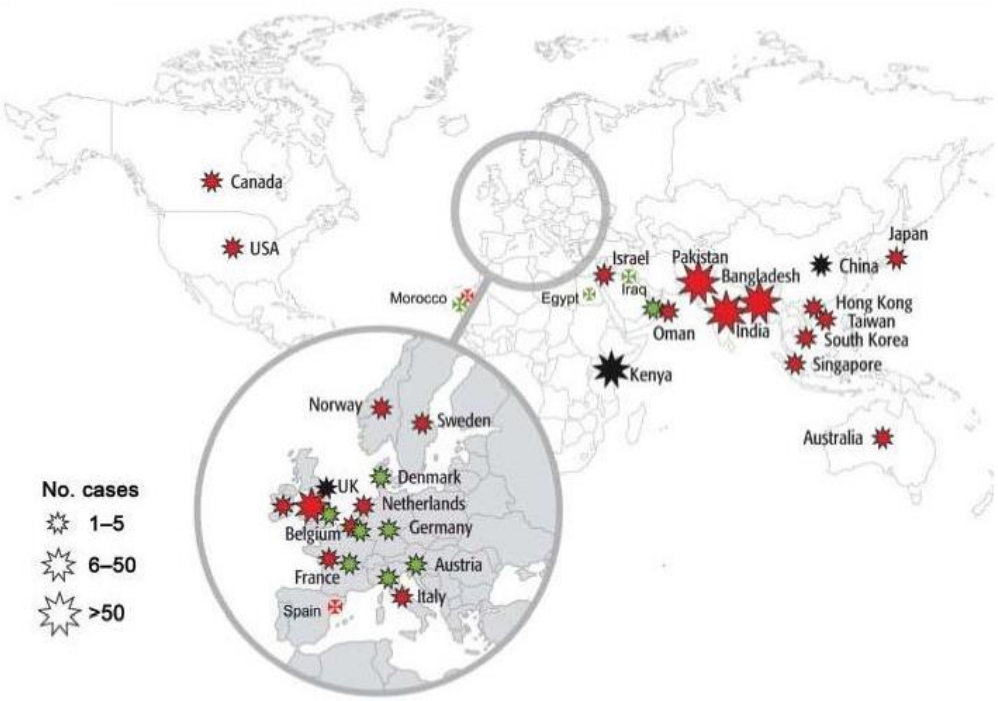


Figure 18. Geographical dissemination of NDM-1 reported cases as of July 2011. The number of cases is shown by the star size, and the star colour refers to the origin of the infection (red= India/Pakistan/Bangladesh; green=Balkans; black= unknown). From [93].

Gram(-) bacteria, in addition to other classes of antibiotics (C3G, carbapenems) [5]. Both these examples, as well as the map in Figure 18, demonstrate that the world is very connected from a microbiological perspective: international transport systems could even be considered a fourth mode of gene transfer, alongside transformation, transduction and conjugation.

Bacteria readily exchange genes in nature, and antibiotic resistance has allowed us to see how extensive these transfers can be. Bringing all these notions together and applying them to a clinically-relevant case, the most probable scenario to explain, in a patient, a *de novo* apparition of resistance in an infectious strain involves commensal bacteria. Indeed, the number of bacteria at the initial infectious site is generally small, hence not opportune to mutant selection. The current theory depicts a two-step mechanism: first, the antibiotic, while in the gut and because of its effect on the gut flora, selects for resistant (naturally or acquired) commensal bacteria; then, in contact with this surviving species, the infectious strains will be able to acquire their resistance genes by means of horizontal transfer. This mechanism would also explain how resistance develops between two courses of antibiotic treatment, where the infectious bacteria have become resistant to the antibiotic they were previously susceptible to (during the first episode of care). It also ensues from this theory that antibiotics with elimination by the biliary tract, hence with a prolonged contact with the gut flora, would be the category that mostly selects for resistant bacteria [94].

c. How does resistance operate?

These genetic mechanisms, once obtained, can serve resistance purposes in various ways. It is often described that a bacterium would resist the action of an antibiotic by four typical mechanisms (Figure 19).

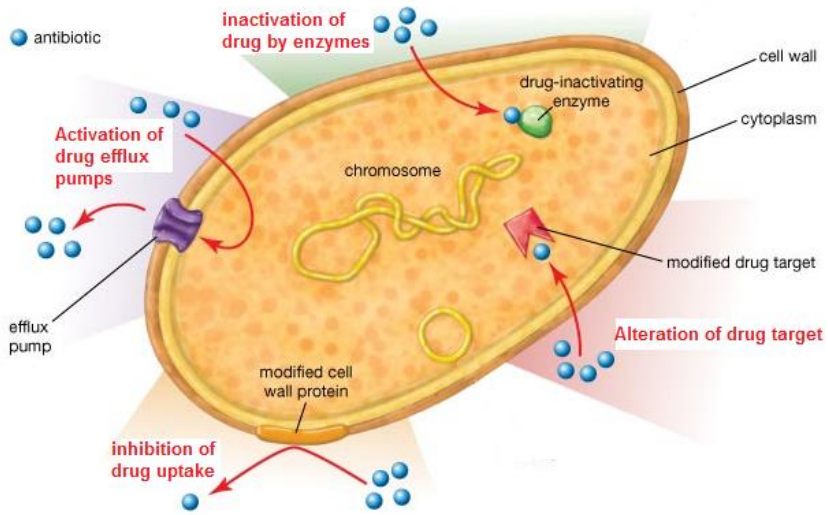


Figure 19. Main types of antibiotic resistance. Adapted from Encyclopaedia Britannica, Inc. (2009).

First, in a rather non-specific way, it can prevent the entry of the drug, for instance by expressing a modified uptake membrane protein (for antibiotics in Gram(-) bacteria requiring porins, such as imipenem in *P. aeruginosa*), or similarly expel it actively, via pumps (a great variety of pumps exist in *P. aeruginosa* or *E. coli*). Other mechanisms are drawing on specific tools such as enzymes that would inactivate the antibiotic (such as penicillinases and penicillin), or that would in a similar fashion modify the antibiotic's target, either creating a new one (such as with methicillin and the PBP-2a in MRSA), enzymatically modifying the original target (such as the *Staphylococcus* methylase and erythromycin's target, a particular nucleotide in the 23S rRNA), or even increasing the number of target's copies (*Enterobacter* and trimethoprim), to cite a few. Examples of resistance mechanisms according to antibiotic classes are provided in Appendix 4.

Yet resistance is not only a natural mechanism described above for the sake of admiring a microbiological tour de force; it also has, at the macro level, worrying features – with implications for many stakeholders including industrials (concerned with new antibiotics R&D), clinicians (concerned with treatment failures) and politicians (concerned with public health) –, that are the topic of the next passage.

B. THE WORRYING VERSATILITY OF RESISTANCE

a. Resistance is a natural (and ancient) phenomenon

Resistance predates the modern selective pressure introduced by our clinical antibiotic usage. Because of, first, such a diversity of resistance mechanisms that have evolved to target almost all available drugs and, second, a worldwide dissemination that has happened over the last several decades, a contemporary emergence of resistance is very unlikely : the phenomenon must have its roots deeper in the bacterial history [95].

It is actually easy to understand and explain the ancient nature of resistance by going back to the origins of the compounds. Most of the available antibacterial drugs have been extracted from natural sources and are therefore products of the microorganisms themselves. Considering the ancient origin of bacteria, it is no surprise that, having been exposed to these lethal components for such a while or needing to protect themselves from their own production, bacteria have sought mechanisms to defend themselves, and antibiotic production could be as old as them. Indeed, studies have estimated the origin of natural antibiotic compounds to range from 2 billion to 40,000 years ago, and the DNA resistance sequences from permafrost sediments dated about 30,000 years confirm this hypothesis: for instance, among the retrieved and analysed sequences, the β-lactamase genes showed between 53% and 84% amino-acid identities when compared with the current determinants ; even the purified enzymes synthesised from resistance sequences show similar quaternary and tertiary structures, emphasising their functional form thousands of years before clinical use of antibiotics [96].

Antibiotic resistance did not appear plainly to us before the clinical use of the drugs, and even when entering the golden age of antibiotics were we warned from the very inception that the story of antibiotics would go hand in hand with the story of resistance. While working with his dyes and trypanosomes did Ehrlich and his postdoctoral colleague witness the first example of the emergence of resistance to chemotherapy [97]; further with the sulfamides experience did antimicrobial resistance emerge on a widespread scale [98], and Fleming confirmed the phenomenon with his morality tale at his Nobel prize speech. Indeed, penicillin, which was more effective than sulfamides on staphylococcal strains, became almost useless against these species by the late 1940s as near 100% resistance rates were observed [99]. What is more is that in the early 1950s *Staphylococcus* spp would become increasingly resistant to novel broad-spectrum antibiotics too, and the scientific community would face a completely new and poorly understood phenomenon.

A tale of a species' adaptability

1940: Penicillin is made commercially available, particularly to treat *Staphylococcus aureus*.
1944: The first detection of a resistant *Staphylococcus* strain is reported [100]; an enzyme, called β-lactamase for it hydrolyses the lactam core of the drug, is responsible for the drug's inefficacy; meanwhile, in 1950, in Paris and London, 50% of *Staphylococcus* strains are resistant to penicillin[12].
1960: An antibiotic resistant to the action of the β-lactamase, methicillin, reaches the market.
1961-62: The first methicillin-resistant *Staphylococcus aureus* species (MRSA) are identified in France and the UK [101], but it is not before the 1980s that the outbreaks spread globally. This time, bacteria resist the new antibiotic by a mechanism different from the first one: they modify the drug target.
1981: Vancomycin, discovered earlier on and active on MRSA strains, is more and more used; an inhibitor of β-lactamase, clavulanic acid, is brought to market in association with a penicillin-type antibiotic (ampicillin).
1996-97: An intermediate vancomycin-resistant strain is identified (called VISA, for Vancomycin-Intermediate *Staphylococcus Aureus* [102]; 2002: a completely resistant strain emerges: VRSA, for Vancomycin-Resistant *S. Aureus* [103]. The bacteria have found yet another target-modifying resistance mechanism, the third one. Between 1997 and 2004: identification of 30 VISA cases and 3 VRSA cases.

The evolution and sequence in the development of resistance from *S. aureus* is a telling example of another worrying characteristic of resistance, namely the continuous back-and-forth movements and adaptation between the drugs and their targets. Strains initially resisted β-lactams (penicillin) by producing a β-lactamase, an enzyme secreted in the extracellular space to destroy the antibiotic and encoded on a *bla* gene. It is very likely that this gene have initially originated in the soil bacteria to combat naturally-produced penicillin (since the drug comes from a natural source) [104] and its plasmidic localisation would explain its widespread diffusion among other species. To counter this resistance mechanism, scientists have developed a new drug, methicillin, with a bulky side chain to substantially slow the hydrolytic step of the antibiotic–β-lactamase complex, making it useful to treat penicillin-resistant strains [59]. But bacteria fought back: this time not by developing another enzyme to destroy the drug, but by modifying the target it binds to. The traditional penicillin-binding proteins (PBPs) are replaced by a variant, PBP-2a, encoded on the *mecA* gene [105]. Hence, methicillin is not effective anymore. Where did scientists go from there? Attempts have been made to develop drugs that would target PBP-2a [106], but the therapeutic solution came from a drug that

[12] Today, virtually all *Staphylococcus* species are resistant to penicillin [99].

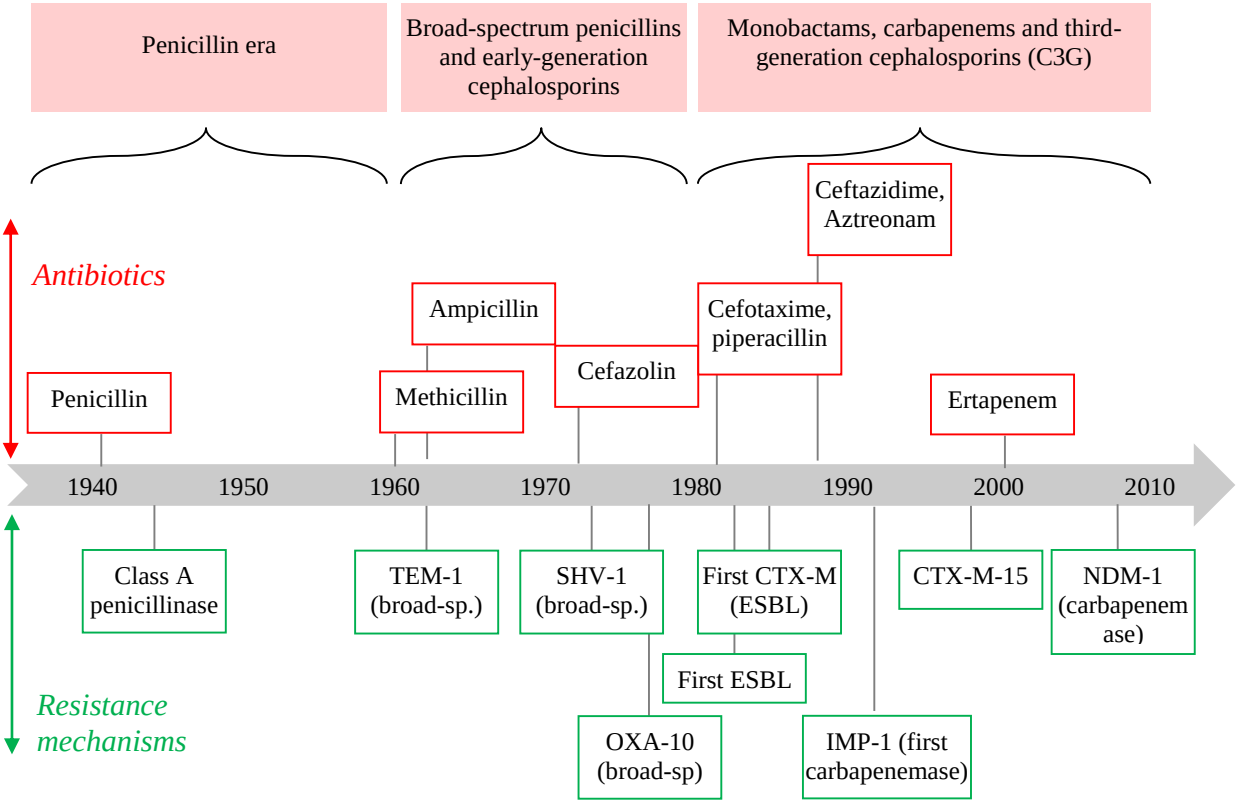
was approved at the same time as methicillin, but barely used because of its toxicity profile[13]: vancomycin. Whereas it had been cautiously used since the 1960s, resistance did not develop until its more widespread utilisation, hence the 30-year window for clinical resistant outbreaks to appear. Thanks to the *vanA* gene of plasmidic origin[14], bacteria were able to modify the drug's target (the pentapeptidic part of the peptidoglycan).
Nowadays, the remaining therapeutic solutions consist of a few costly drugs, such as linezolid or daptomycin; worryingly, resistance to both drugs has already been reported [107], [108].

The resistance development profile of *S. aureus* is also meaningful as an illustration of resistance, not only for the 'continuous battle' tale, but also due to the bacterial character, *Staphylococcus aureus*. One of the most mediatised bacteria ('the golden staph', 'the superbug MRSA', among other nicknames), they are very widespread (healthy colonisers are plentiful, on external surfaces and natural orifices); among the leading causes of bacteremias and surgical wound infections, they are responsible for a broad range of infections, from the most common to the most severe (and some strains can have a virulence factor, the Panton-Valentine leukocidin, or PLV toxin, rare but of dangerous and impressive consequences); and they are difficult to get rid of, because of their multidrug-resistance profile (MRSA, VRSA).

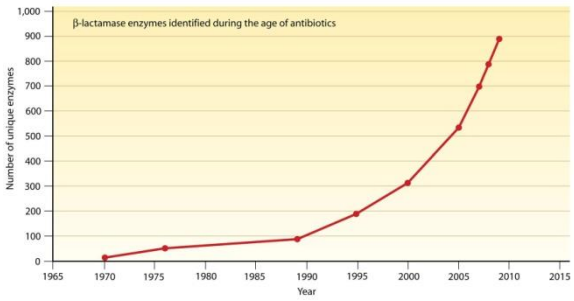
An illustration of the ever-changing resistance solutions

Other examples of sequential development of resistance mechanisms to counter regularly-introduced drugs are well known, illustrating the continuing, ever-changing characteristic of resistance, such as the evolution and growth of the β-lactamases families of enzymes (Figure 20): penicillinases, cephalosporinases, carbapenemases, then derepressed enzymes and extended-spectrum β-lactamases (ESBL) populate a global, 200+ enzymes set, divided into four classes, that has spurred on the pharmaceutical R&D but whose identification in virtually every species poses a real therapeutic threat [104].

[13] Ototoxicity, nephrotoxicity and its IV route of administration made it a last-resort antibiotic.
[14] Acquired from *Enterococcus* spp (see Paragraph (e) below).



A.



B.

Figure 20. A: Evolution of β-lactam antibiotics and β-lactamases. TEM-1 is active against aminopenicillins and the early cephalosporins; ESBLs hydrolyse the majority of penicillins and cephalosporins; OXA-10 is effective against the broad-spectrum penicillins, C3G, and aztreonam; IMP-1 is a metalloenzyme conferring resistance to all β-lactams except aztreonam; CTX-M-15 (an ESBL) additionally confers resistance to ertapenem; NDM-1 confers resistance to virtually all β-lactams (variable susceptibility to aztreonam). From [109] and [110]. B: Explosion in the number of identified β-lactamases. From [111].

c. Resistance can be multi-purpose

An opportunistic bacterial Gram(-) pathogen, *Pseudomonas aeruginosa* is seldom part of the normal microbial flora in humans. Colonisation may, however, exceed 50% during hospitalisation, and infections lead to pneumoniae, urinary tract infections or bacteremia to name a few, especially in intensive care units (ICUs), where antibiotics ineffective against *P. aeruginosa* significantly increase the risk of colonisation or infection [112]. This is an especially problematic matter: the species is naturally resistant to a lot of antibiotics, making the care management of seriously ill

patients in ICUs quite tricky. And it is quite remarkable that resistance to a variety of structurally different molecules is conferred by a single resistance mechanism: efflux pumps.

Efflux pumps are categorised in five superfamilies, based on their substrate specificity, their energy source and their sequential identity. A pump is organised as a tripartite system, with a cytoplasmic membrane transporter, a periplasmic membrane fusion protein, and an outer membrane porin, forming a channel from the intracellular compartment to the extracellular environment. The largest number of pumps in the bacterial strain belong to the RND family, with for instance the proteic complex MexAB-OprM which, with its broadest substrate profile, enables the bacterium to extract β-lactams and their inhibitors, fluoroquinolones, tetracyclines, chloramphenicol, macrolides, sulfonamides, trimethoprim, fusidic acid or even rifampicin [113] (see the efflux of fluoroquinolones in Figure 21). It is constitutively expressed in wild-type cells, hence the intrinsic resistance profile (but with an expression dependent on growth-phase quorum sensing, through a MexR regulator [114]). An additional type of efflux system that takes charge of aminoglycosides has also been identified [115], leaving an even thinner effective therapeutic arsenal.

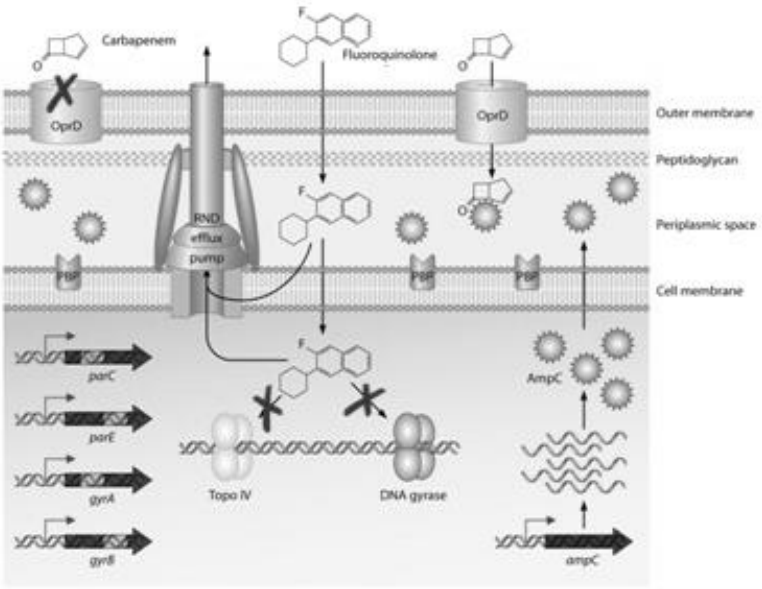


Figure 21. Efflux of fluoroquinolones, that initially pass through the outer membrane passively, by the chromosomally-encoded efflux pumps, such as the RND pumps pictured here; antibiotics that require a transporter to enter the bacterium in the first place are exposed to another risk of resistance, by decreased production or loss of function of their OprD protein. From [116].

The efflux pumps are a very polyvalent family of molecules, highlighting the challenge that therapeutics face when in front of bacterial strains thus equipped: the typical "one enzyme, one substrate" rule does not apply here, and by displaying such a non-specific resistance mechanism, *P. aeruginosa* has disrupted the rules. With such versatility, the efflux pump mechanism of resistance is a defy to antimicrobial drug discovery. One of the main therapeutic ideas was to develop

inhibitors of efflux pumps [117], but so far none has reached the clinic, making the rationale of their druggability quite insubstantial.

As a side note to add to the challenge of intrinsic non-specific resistance, *P. aeruginosa* also has specific, natural mechanisms (for instance, the inducible AmpC cephalosporinase encoded by the *ampC* gene, or the porin OprD-mediated resistance in its outer membrane, Figure 21) as well as an ability to pick acquired mechanisms, making it an even tougher target to reach (β-lactamases from the class A serine β-lactamases family, metallo-β-lactamases against carbapenems, aminoglycosides-modifying enzymes...) [118], [119].

Multidrug-resistant phenotypes are a real challenge, and potential imports of additional resistant genes, a continuing threat: the CDC has estimated that 70% of bacterial infections are caused by bacteria resistant to more than one antibiotic [120]. While a highlight of the impressive ability of *P. aeruginosa* to develop resistance at a low cost, this paragraph has emphasised a mechanism that seemed inexplicable due to the variety of ineffective antibiotics. Bringing new classes of antipseudomonal drugs is a pressing need, but while very few are in clinical development or close to reaching the market, therapeutic strategies are turning to combinations of antibacterial agents or further optimisation of current antibiotics.

d. Resistance can be a double-edged sword

At the root of any resistance mechanism is the very concept of mutation, translating in the fact that a bacterium has to undergo some changes in its structure to continue to survive. In some cases, these changes are hardly inconsequential, and the mutation may impose a fitness cost on the species [121]. If the fitness cost is low, resistance (and the mutation) can persist and be passed on to the next generation; this is the case for example for *gyrA* and *parC* mutations in *E. coli* (Figure 22, blue dots). On the other hand, if the burden is high, the negative consequences may prevail over the edge gained by defeating the antibiotic; a major reduction in fitness occurs in *E. coli* with mutations in the *marR* locus, isolated or associated with *gyrA* mutations (Figure 22, red dots).

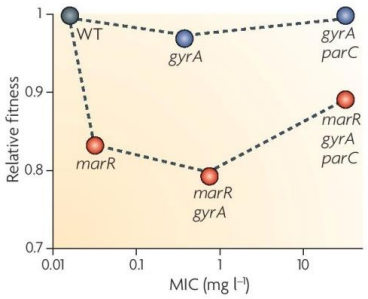


Figure 22. Impact on E. coli*'s fitness of mutations conferring resistance to fluoroquinolones. From [121].*

In a study analysing 30 resistance mechanisms across fifteen species, 16 were associated with a significant fitness cost, defined as an increase in multiplication time [121]. An example of such a fitness cost is the resistance mechanism of *S. aureus* (MRSA) and its unique, new enzyme, PBP-2a, encoded by the *mecA* gene and replacing the traditional ones. The transpeptidase activity of PBP-2a is weaker compared to native PBPs and requires the transglycosylase activity of PBP-2. This burden might explain why the synthesis of PBP-2a is inducible, why native strains select against the expression of PBP-2a and why some resistant strains can lose their *mecA* genes [122].

In other circumstances of a fitness burden consequent to resistance, some ingenious species have developed compensatory secondary mutations and mechanisms. This is, for instance, the case with *P. aeruginosa* and its gyrase mutation that permits resistance to fluoroquinolones [123]: supercoiling was guaranteed and restored to normal levels due to additional mutations (though not located in any of the genes expected to alter supercoiling, such as *gyrA-B, topA, parC-E*). Another compensatory mechanism is the amplification of a compound activated by an enzyme, target of the antibiotic: this is the case in *Salmonella* spp whose deformylase activity, involved in peptidic translation initiation, targeted by deformylase inhibitors, is compensated for by the rise in tRNAi (its product) levels [124].

e. Resistance spans across sectors

Vancomycin resistance took the clinical community by surprise when it emerged in pathogenic enterococci in the late 1980s. *Enterococcus* spp are serious Gram(+) pathogens, commensal hosts of the gut and frequently isolated in nosocomial infections such as endocarditis, bacteremia, infections of the urinary or biliary tract, among others; *E. faecium* and *E. faecalis* are the most commonly isolated strains. These bacteria are of concern to the healthcare professionals because of their intrinsic resistance, equipping them with genetic materials present in the genome of all these species, enabling them to escape several antibiotics: β-lactams and cephalosporins, aminoglycosides, lincosamides, streptogramins and trimethoprim-sulfamethoxazole [125]. For instance, their constitutive expression of chromosomally-encoded, low-affinity versions of transpeptidases/transglycolysases responsible for the peptidoglycan wall synthesis (also referred to as Penicillin Binding Proteins, PBPs), PBP5 in *E. faecium* and PBP4 in *E. faecalis*, results in a very low susceptibility to β-lactams and cephalosporins by a weak binding between the drugs and the modified targets. Intrinsic also are the mechanisms leading to aminoglycoside resistance, whether they are expressed through a low cell wall permeability or the production of an aminoside-modifying enzyme (AME), Aac(6')-Ii, ubiquitous among *E. faecium,* that targets tobramycin and kanamycin. Additionally, *E. faecalis* harbours a *lsa* gene, whose expression, with the production of an efflux pump, results in its intrinsic resistance to clindamycin, quinupristin, and dalfopristin [126].

The natural resistance of *Enterococcus* to multiple antibiotics is well documented; in many cases, vancomycin is one of the last therapy that has kept its efficacy. Yet this already large resistance profile became even more worrying with the emergence of additional resistance through acquired mechanisms, one of which coming from a source that had never been reported before.

Since the 1960s-1970s, supplementing food-producing animals with antibacterial drugs to enhance growth had been common practice and had represented the main usage of the antibiotic production. The mechanism behind this growth-promoting use is not clear; it has been said that antibiotics, often used at subtherapeutic doses, might inhibit susceptible bacteria in the animals' guts, reducing both the normal and the pathogenic flora. Avoparcin is one of such antibiotics. It was approved for growth promotion in Europe in 1974 and has been widely used in many European countries, especially Denmark, but never in the US [127].

Yet, in 1988, the first outbreak of infections due to vancomycin-resistant *Enterococcus* has been reported [128]. The particular feature about these infections was that, although some were arising in patients previously treated with vancomycin (in the case of infections to *Staphylococcus* species that antedated the infection at hand), in some patients, the resistant bacteria were not linked to a patient's history of vancomycin therapy. How could it be the case that a bacterial strain was resistant to a drug it had not been in contact before within the host, when intrinsic resistance was not an option? Curious about this oddity, Bates and his colleagues broadened the search and found that the missing link was among farm animals: the resistance to vancomycin had an animal origin [129]. They noticed that the very livestock treated with avoparcin, poultry and chicken, was shown to carry *Enterococcus* strains that were resistant to vancomycin: here was the origin of resistance. Indeed, a sustained contact with the drug at low doses had selected for resistant bacteria, which exhibited mechanisms making them capable of surviving despite the antibiotic. Then, the animals, going through the food chain, can either release their commensal bacteria in the environment or pass them on to humans through their meat; Figure 23 gives an illustration of the various ways bacteria can get from livestock to humans. When examining the links and chains between animals and humans, it became evident that antibiotic-resistant food-borne bacteria could impact human health, whether directly (bacteria of animal origin reaching a human host and causing infections) or indirectly (opportunistic resistant bacteria that would use their host as a vector to colonise another set of patients, such as a hospital setting, or that would exchange their genetic materials with other bacteria, such as with *E. coli* or *Enterobacteriae*).

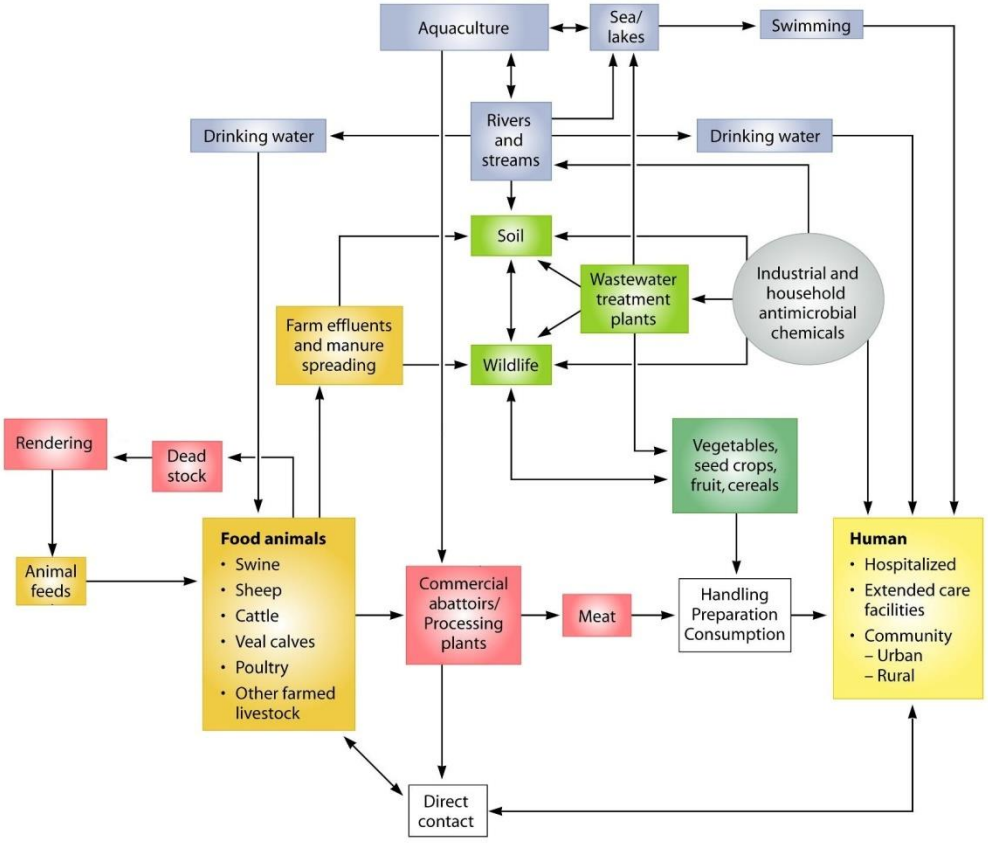


Figure 23. The spread of bacteria through agriculture and other reservoirs (aquaculture, market gardening) to humans in different settings. Adapted from [111].

This cycle explains how avoparcin-resistant bacteria have been able to colonise humans. Yet the patients in question displayed resistance not to avoparcin, which is not in use in humans, but to vancomycin. The explanation lies in the chemical structure of the drugs (Figure 24): while they both belong to the glycopeptide family of antibacterial drugs, their similarity is even more striking. It is not surprising that the resistance mechanisms developed to escape avoparcin worked very well with vancomycin too [130]. Resistance is acquired by a plasmidic transposon, *Tn1546*, identified in *E. faecium* [131], which contains a *vanA* gene that encodes an enzyme (ATP-ligase), working in concert with a *vanH* gene (synthesis of D-Lac from pyruvate), both responsible for producing a modified antibiotic target. The last amino acid in the peptidoglycan needed for cell wall synthesis (usually D-Ala) is replaced by D-Lactate[15]. Chemical variations in Lactate compared to Alanine (notably the loss of the amine group) result in the loss of a key hydrogen bond with the drug, wich leads to a reduced affinity of up to 1000 times.

[15] It is worth noting that this modified target is successfully taken through to the next synthesis stage (lipid II), resulting in a mechanically sound peptidoglycan layer : no fitness cost results from the resistance.

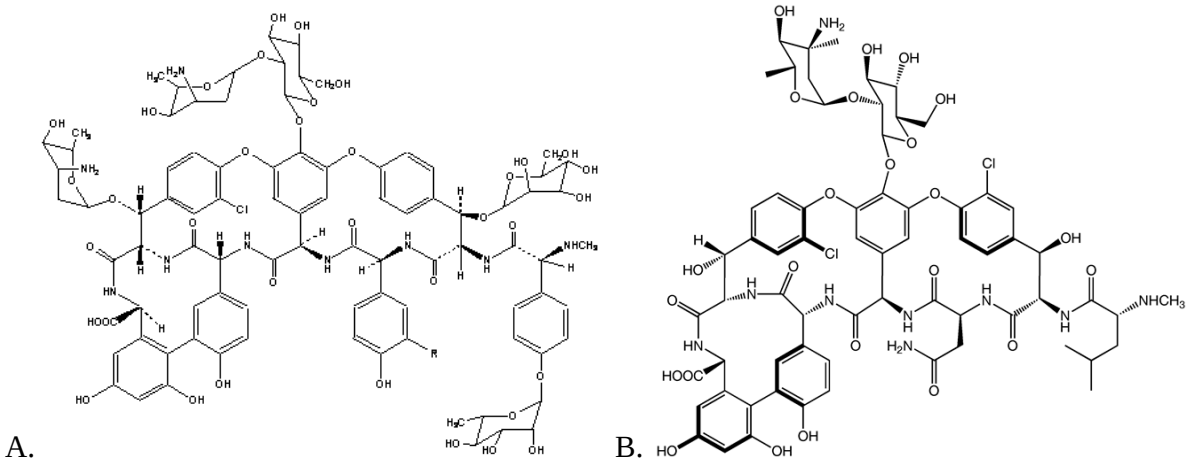


Figure 24. Comparison of the chemical structures of A: avoparcin, B: vancomycin.

Because of public health concerns about resistance to glycopeptide antibiotic drugs, avoparcin was banned in Denmark in 1995, without significantly impacting the sector's productivity [132]. In 1996, Germany took a similar step, and finally in 1997, avoparcin was banned in all EU member states [133]. The results were mixed: while the rates of fecal VRE in poultry decreased significantly, the prevalence in samples from poultry farmers did not decline [134].

This example has highlighted the public health impact that growth-promoting use of antibiotic for livestock production can have, which is a sizeable issue even now. The agricultural sector, for it makes a widespread and sometimes inappropriate use of antibiotics, is therefore a reservoir of antibiotic-resistant bacteria that can affect the public health. This animal reservoir can accelerate the spread of resistant strains, but is not a compulsory stage in the dissemination of resistance: although they had never used avoparcin in animals, the US identified their first case of VRE in 1989 in New York hospitals. The cause for resistance was supposed to be a more common one, surely related to the large utilisation of vancomycin in prophylaxis protocols [135].

SECTION 4. WHERE DO WE GO FROM HERE? THE OVERARCHING FRAMEWORK TO CURB ANTIMICROBIAL RESISTANCE

Mankind is playing an essential role in selecting resistant microorganisms and helping them thrive, whether it be by the mere clinical use of antibiotics, appropriate or not, or by their extensive use in agriculture and animal husbandry, which represents a sizeable portion of the total consumption. Surprisingly enough, the US direct about 70% of their total consumption of antibiotics to the livestock sector [136] (which represents 1.5 times more quantity than the heaviest European user, Spain), when China, whose pork production represents almost 30% of the world's output, consumes

four times more antibiotics than the US for the same quantity of meat [137] and the trends in demand for meat are likely to sustain, if not increase this antibiotic usage. Small outbreaks of hard-to-treat, resistant or multi-resistant infections have surfaced in many parts of the world, in the community as well as in hospitals. These situations are no outliers, but it is the role and responsibility of the scientific corps and the involved stakeholders to prevent them from reaching bigger proportions.

A. A FEW EXAMPLES FOR A COURSE OF ACTION

Many thorough and better-documented reports have addressed the issue of antibiotic resistance with the lens of potential actions and solutions to go forward [138], [139], [140]. It is not the aim here to discuss and analyse all proposals since the focus is primarily on a unique one: the discovery of new antibacterial drugs and the need for a supportive environment (developed in the rest of this thesis). It is however important to remind ourselves that this is far from being the sole way to go, and the aim of this section is hence to put this answer into perspective before delving deeper: the conservation of the current antibiotics is as important as the development of novel ones.

As previously stated, targeting antibiotic resistance requires a multi-disciplinary, cross-sector and international approach, within and beyond the healthcare setting (Figure 25).

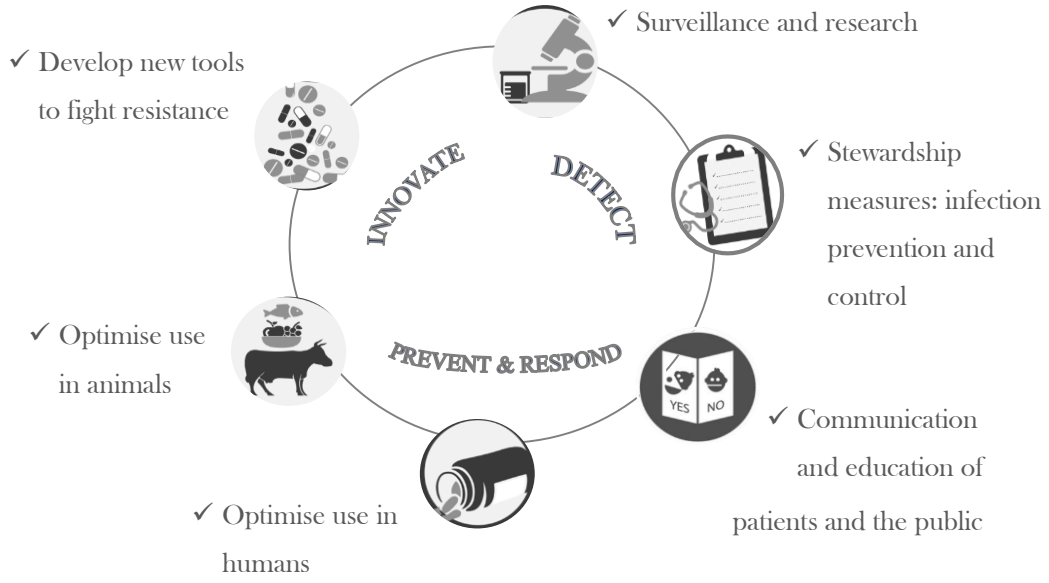


Figure 25. Core actions to fight antimicrobial resistance. Adapted from WHO [14] and CDC.

Understand, educate, integrate

As within any combat, intelligence is paramount. Governments and private sectors have taken the lead on several ongoing surveillance systems on bacteria and resistance, as well as on antibiotic use and efficacy (examples, such as EARS or SENTRY, are provided in Appendix 5, Tables 5-6); organised and comprehensive surveillance data will help guide healthcare strategies and inform public policies. A national leadership with a strong political commitment is also necessary to promote a rational antibiotic use in hospitals as well as in the community. It is an undeniable fact that preventing a broad usage of an antibiotic will delay the emergence of resistance (as evidenced by vancomycin and its 30-year resistance development window): stewardship and appropriate use of antibiotics is indeed one of the biggest challenges facing policies targeted at bacterial resistance. Furthermore, the education of patients and the general public, and the change of social norms are also crucial to prevent infections in the first place, and to ensure the right use of antibiotic therapy when prescribed.

Beyond use in human beings, a holistic approach (the "one health" approach) will help safeguard the future of antibiotics: a broader ecological stewardship programme must address the use in the agricultural sector, since resistance features can transfer from animals to humans, or the resistance dissemination through antibiotic pollution of waste waters [141]. Yet such a discussion with other actors will unveil another wide range of issues, such as, for the agricultural sector, concerns about the economic livelihood of meat producers, the wellbeing and health of animals or even the resulting negative publicity for raw products, more subject to containing enduring bacteria... To start the ball rolling on such discussions, the WHO has issued specific actionable tasks targeted at each of the involved stakeholders group: (see Appendix 5, Figure 4).

Any use of an antibiotic, whatever its therapeutic class, will do its share of selecting resistant bacteria, whether agricultural, piscicultural [142], veterinary, human, prophylactic, curative... It is therefore important that guidances of appropriate use target each and every single usage of these drugs.

Actors and governance

As the required measures span across several, diverse sectors, with different (maybe opposed) interests and challenges, their viability is dependent upon a strong governance role by a capable institution taking charge of the oversight of the implementations. Global institutions such as the WHO may be seen best placed for this role, having the best recognition and capacity to connect all the elements that revolve around the topic of antibacterial resistance. But these institutions and their peers – e.g. governments, NGOs, public sector agencies – are often prisoners of the inertia resulting from administrative, bureaucratic or diplomatic issues. However, other stakeholders can have an

influential power that should be reckoned with, and a recent campaign could lead to very concrete results in the domain or agricultural antibiotic usage, on a scale worthy of the WHO's. In a letter dated of March 2016, a coalition of more than fifty institutional investors, managing a total of $1.4 trillion in assets, asked the ten biggest American and English fast-food groups, including the likes of McDonald's, JD Wetherspoon or Domino's Pizza Group, to set a timeline to cease to use meat from antibiotics-fed animals [143].

Could real and practical solutions to the current resistance crisis come from the financial world? Finance certainly has the muscle to strongly influence multinational companies, yet one has to be careful that their interest in this area aligns with public health interests. The move is understandable on the part of the investors since, with the growing public and political awareness (see Section 1) and the regulation likely to become tougher on one side, and the fact that these large companies are key to the institutional investors' portfolios on the other side, this demand results from a proper risk-management strategy. Such proactive recommendations are very timely considering that the FDA is taking action on the matter. The 2015 Veterinary Feed Directive (VFD) final rule already put a more stringent set of regulations on veterinarians' usage of antibiotics in animals [144], while by December 2016 the full implementation of its Guidance #213 will make use of antibiotics for growth promotion illegal [145].

B. DISCOVERING NEW ANTIBIOTICS

Regardless of all the stewardship and guidances for a more rational usage of antibiotics, just alluded to in the previous paragraph, resistance will eventually develop, rendering the available drugs ineffective. This explains why, in addition to ensuring an appropriate conservation of existing drugs, bringing new antibiotics to market, and especially new classes of antibiotics, is of utmost importance. During the golden era of antibiotics, it was a belief firmly held that the scientific and industrial community could bring a technological fix to the race against resistant organisms; nowadays, a more common view is that the industry is helpless in front of the more and more complex and widespread resistance mechanisms. The reasons underlying such a disinterest in the field are linked to the economics and business model of antibiotics; they are reviewed in Part 3. Nevertheless, it is essential to keep in mind that the fix has not been provided and that novel drugs would still be welcome. Analysed in the next sections are the options that could be put forward to nurture antimicrobial drug discovery – whether scientific, economic or regulatory.

PART 2: THE SCIENTIFIC CHALLENGES TO THE ANTIBIOTIC INDUSTRY AND THE 2016 WAY OF DISCOVERING NEW ANTIBIOTICS: AN ILLUSTRATION WITH TEIXOBACTIN

In these times depicted by some as a dreadful "post-antibiotic era" with a "drying pipeline", there seems to be a significant strain on the research and development of innovative antibiotics. In this context, the discovery of a molecule with a new mechanism of action, teixobactin, is very timely – and so seems the claim of 'no resistance' that accompanies its introduction. What are the objective scientific challenges that are associated with antibiotic drug discovery? Is such a pessimistic view justified on scientific grounds? Can teixobactin really get away with this 'no resistance' catchline?

SECTION 1. AN APPROACH TO ANTIBIOTIC DRUG DISCOVERY

Talking about antibiotic drug discovery, or the scientific challenges to find such new drugs, lays the way open for an assessment of nearly all the drug-making processes: there is not a single approach that has not been tried to antibiotic drug discovery, from natural product screening to genomics or use of viruses, not to mention monoclonal antibodies. Yet very few novel compounds have successfully been developed, and to apply the even more stringent lens of novelty with new mechanisms of action, there have been no new antibiotic classes after the oxazolidinones (1978)[16] or the lipopeptides (1987)[1] classes. This begs the question: is the science behind antibiotics a real obstacle to new drug developments?

A. WHAT DOES IT TAKE TO FIND A NEW ANTIBIOTIC ?

A company that is willing to discover new treatments against bacterial infections can organise its search in two steps (Figure 26). First, it selects the approach to the infectious disease treatment it wants to take. The most common approach, as within any other therapeutic class, is to find a compound that relieves the patient from their disease, by directly acting on the responsible agent, hence by killing the bacteria: this is an antibacterial compound. Yet there is also a second possible approach, less conventional and not universal, but made possible by the very particularity of the anti-infectious therapies, namely the resistance problem, to turn it into an opportunity: instead of designing antibiotics *de novo*, the aim here is to design compounds to overcome resistance, and

[16] Dates refer to reported initial discovery or patent.

employ a combination approach with existing antibacterial drugs. Therefore, in parallel to "antibiotic drug discovery", this could be viewed as "antiresistance-to-antibiotic drug discovery". These 'antiresistance' compounds have an indirect antibacterial action, since they are supplementing antibiotics and not killing the bacteria by themselves.

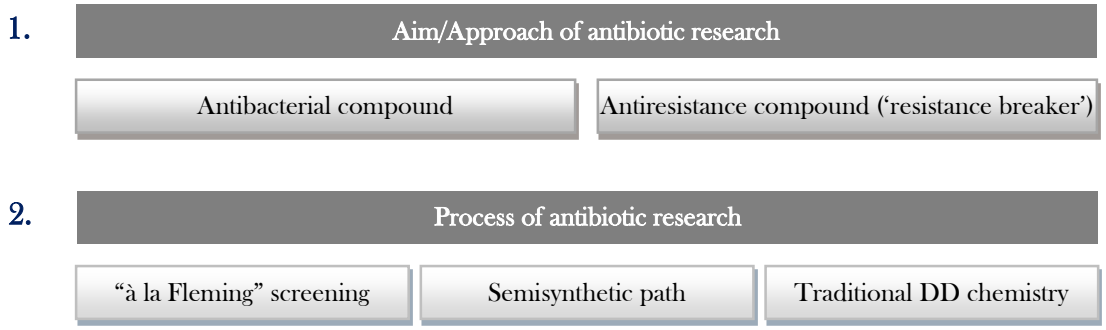


Figure 26. Organisation of drug discovery in the antibiotic industry; DD= drug discovery.

There are also other approaches to treating infectious diseases that could be followed, but they are not treated here as they are still in their infacy (for instance, harnessing the normal microbiota to fight infections, suppressing pathogenic behaviour or manipulating bacterial signaling and communication[17] [146]).

Regardless of the aim of the final compound, and in a second time, the company chooses the drug discovery path it wants to follow. Here again, the antibiotic field offers a variety of possibilities, whether it be the historical route of natural screening, the traditional medicinal chemistry process, or a hybrid between the two, a semisynthetic path to optimise natural leads.

With what looks like twice as much R&D opportunities compared to other therapeutic areas, with the choice between discovering a conventional antibiotic or an antiresistance compound, how can it be explained that antibacterial R&D is stalling, and what are the real scientific requirements to make antibiotic drug discovery thrive?

a. New antibacterial compound research

Most of the antibacterial compounds on the market as well as in the development stages are small molecules that target elements of the susceptible bacteria, enzymes or nucleic acids or other components essential to the physiology and viability of the bacterial cell (by contrast with elements

[17] An antibacterial agent that does not kill is also less likely to induce selection pressure, and hence to select for antibiotic resistance. Yet these agents are not easily found by drug discovery techniques that focus on inhibition power on essential biomolecules.

from their defense and resistance mechanisms). Also, because many exploitable targets have already been used, the bulk of recent or forthcoming antibiotics represents mainly incremental variants of existing drugs. Yet it is important to question the capacity of this approach to bring to market drugs that respond to public health needs: is this the best allocation of efforts and resources to antibacterial drug discovery?

The view defended here is that this current production rationale is not ideally suited to the production of the much-needed types of antibacterial drugs. Expanding existing families such as cephalosporins whose broad spectrum of action is viewed as a plus when it comes to commercial opportunities does not provide an answer to the pressing medical needs. As described in Part 1, agents targeting Gram(-) species are much awaited, especially against species such as *P. aeruginosa, Campylobacter* or *Enterococcus* spp which have acquired redoubtable multi-resistance mechanisms. Additionally, rational antibiotic stewardship commands that we develop narrow spectrum rather than broad spectrum drugs, as broad-spectrum agents, which are by definition more used and circulate more into the environment, hasten the development of resistance. Cephalosporins provide again a telling example since their use has given rise to a prolific family of resistance enzymes, a family whose size can only compare with that of the cephalosporin compounds. Using a narrow spectrum agent amounts to lessening the diffusion of the selection potential.

There is a particular implication from this rationale though. The narrower the spectrum, the bigger (and the more pressing) the need for appropriate diagnosis techniques. Indeed, when drugs are developed to only treat a handful of bacterial strains, empiric therapy based on a hypothetical and inferred species diagnosis will not be a sensible solution anymore. This would call for a broader use of, for example, real-time PCR (with an identification time of less than 30 minutes) not only to identify the bacterium, but also its resistance genes, to help prescribe the most suitable antibacterial therapy[18].

b. New antiresistance, or 'resistance breaker', compound research

The more disrupting compounds in antibacterial therapy are likely to come from antiresistance strategies where, with a change in paradigm from targeting vital pathways to targeting resistance pathways, and with the feeble presence of only one class on the market, most remains to de discovered.

Because the rapid obsolescence of antibiotics is rooted in their mechanism of action with the apparition of resistance, this alternative strategic vision to developing new antibiotics is based on finding compounds able to revert or inhibit the resistance mechanism, and combine them with the

[18] The need for improved diagnostic tools is not the focus of this thesis and several reviews can be recommended for their thorough analyses of the aspects of this particular matter (e.g. [274], [343], [345], [369])

original antibiotic. Since the resistance mechanisms employ tools that are biomolecules (such as enzymes, porins...), the logic would be to block the biosynthesis pathways of these biomolecules: this could possibly take the form of a 'substrate bomb' (for instance, a prokaryotic amino acid). Conversely, a "downstream" approach would more conventionally block the biomolecule responsible for resistance.

This idea has given rise to novel antiresistance classes, or "resistance breakers", whose main representatives are the β-lactamase inhibitors, that are employed in successful combinations with an edge over traditional antibiotics, such as the blockbuster Augmentin (amoxicillin + clavulanic acid), Zosyn (piperacillin + tazobactam) or Unasyn (ampicillin + sulbactam). Another antiresistance class of therapeutic potential but of no current marketed compounds is the class of efflux pumps inhibitors [147], marked by the difficulty in finding molecules that inhibit the multiplicity of pumps that are responsible for the Gram(-) and Gram(+) broad resistance phenotypes and the difficulty in preventing adverse events [148].

This approach with resistance breakers is in theory attractive to the pharmaceutical industry, whose incentive is to develop agents to which resistance is difficult or will be slow to appear in order to extend the commercial life of the antibiotic, but it must not overlook the specific issue to combination therapies, namely the appropriate handling of pharmacokinetics (for instance, the concentrations of both compounds must be superior to the MIC at the same time, otherwise the synergistic effect is lost)[19]. Also, this strategy should not be viewed as a permanent solution against resistance, since it is unlikely that resistance to these "antiresistance" drugs will not appear, as with any compound targeted at bacteria; yet, with its capacity of "buying more time" against resistance, this approach might nevertheless have a substantial edge in that it extend the therapeutic lifetime of a company's antibiotic.

c. Drug discovery routes and their scientific challenges

Natural and semi-synthetic approaches

The discovery of new antibiotics has not always followed the drug discovery rules typical of other therapeutic areas. At the first end of the spectrum (see the second block in Figure 26), identifying compounds can be done following the historical process that Fleming had started the field of antibiotics with (and that Waksman had continued in a more extensive way), namely taking advantage of the paradigm that antibiotics are initially natural products identified in microbial cultures (whether this search is intended for or not). Drawing on the results of this process is the

[19] This discussion can broaden into combination therapy (two antibacterial agents, whether one is a resistance breaker or not) as another solution against resistance, but this approach, that was for instance recommended for artemisinin by the WHO to delay the emergence of antimalarial resistance, is outisde the topic of this thesis; see, for reference, [346], [347]. [348].

second approach, the semisynthetic path, which chemically enhances the natural product, building on its chemical core to optimise its therapeutic potential (broader spectrum of activity, efficacy against resistant organisms, enhanced pharmacodynamics...). Both approaches have indeed brought the vast majority of today's classes to market: with the names of penicillin, streptomycin, cephalosporin, vancomycin, tetracycline or even erythromycin as natural products, and ampicillin, ceftriaxone, tigecycline or telithromycin as hemisynthetic derivatives, 70 out of the 90 drugs marketed between 1982 and 2002 originated in natural sources [149], from various organisms (Table 2): with 8,400 out of the 12,000 known natural antibiotic compounds, the *Actinomycetes* order and especially the *Streptomyces* family are the most prolific producers [150]. Chemical re-engineering has, for instance, had most successes in the β-lactam family, with the development from penicillin G of penicillin V (phenoxypenicillin), resistant penicillins

Original microorganism family	Antibiotics
Fungi	• **Penicillins** (*Penicillium notatum, P. chryzogenum)* • **Griseofulvin** (*P. griseofulvum, P. patulum, P. nigricans)* • **Cephalosporins** (*Cephalosporium salmosynnematum)* • **Fusidin** (*Fusidium coccineum)*
Actinomyces	• **Aminoglycosides: streptomycin** (*Streptomyces griseus),* **tobramycin** (*S. tenebrarius),* **neomycin** (*S. fradiae),* **kanamycin** (*S. kanamyseticus),* **gentamycin** (*Micromonospora purpurea),* **sisomicin** (*M. inyoensis)* • **Tetracyclines: chlortetracycline** (*S. aureofacines),* **oxytetracycline** (*S. rimosus)* • **Chloramphenicol** (*S. venezuelae*) • **Macrolides : oleandomycin** (*S. antibioticus*), **erythromycin** (*S. erythreus*) • **Lincomycin** (*S. lincolnensis*) • **Rifampicin** (*S. mediterranei*) • **Polyenes: nystatin** (*S. noursei*), **levorin** (*S. levoris),* **amphotericin B** (*S. nodosus*) • **Inhibitors of beta-lactamases: clavulanic acid** (*S. clavuligerus*)
Bacteria	• **Polymyxin** (*Bacillus polymyxa*) • **Gramicidin** (*B. brevis*) • **Subtilin** (*B. subtilis*) • **Piocianin** (*Pseudomonas aeruginosa*) • **Sorbistin** (*P. sorbicinii*) • **Monobactam** (*Chromobacterium violaceum*) • **Nisin** (*Streptococcus lactis*) • **Coliformin** (*E. coli*)
Plants	• **Chlorellin** (*Chlorella vulgaris*) • **Allicin** (*Allium sativum*) • **Raphanin** (*Raphanus sativus*) • **Arenarin** (*Helichrysum arenarium*)

Animal Tissues	• **Interferons** (spleen, macrophages, tissue cells) • **Lysozyme** (most body fluids, saliva)

Table 2. Examples of natural antibiotics according to their source. Adapted from [151].

such as methicillin, extended-spectrum penicillins such as aminopenicillins, carboxypenicillins, and ureidopenicillins, and then the broad families of cephalosporins (C1G-C3G), monobactams, carbapenems. Similarly, the aminoglycosides family has benefited a lot from the hemisynthetic capabilities. This approach is currently still the most commonly chosen one, with the consequence that the most recent molecules brought to market, even if classified under new family names, are chemically similar to older families (tigecycline (glycylcycline) is derived from tetracycline, telithromycin (ketolide) from macrolides), with the exception of oxazolidinones and lipopeptides.

Finding natural sources of antibiotics requires the growth of a strain in conditions appropriate to generate the production of antibiotics, followed by the screening of aqueous and solvent extracts of cultures and follow-up chemistry to purify, identify and optimise the isolated compound (Figure 27). Perceived as a more manual method than screening massive compound libraries, natural product lead discovery's major bottleneck is the time required from the extract (hit) to compound (lead).

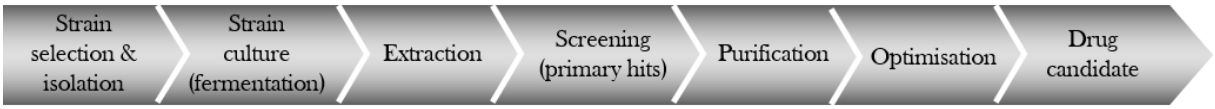


Figure 27. Steps in natural drug screening. From [152].

Although the principles look straightforward, this natural route is peppered with difficulties pertaining to the bacterial culture that emphasise the scientific challenges of this particular drug discovery path. For one thing, the growth conditions of a selected species may not be reproducible in the laboratory, or additionally, a variation in the growth conditions may lead to substantially different secondary metabolites, with variable, if any, antibacterial properties. In this case, the strategy to grow each strain under a small number of different conditions can be adopted. The most recent approach in which bacteria are grown in microplates is another successful solution which has led to the discovery of teixobactin (see Sections 2 and 3). The next critical challenge is the extraction process; the extraction of natural products may indeed be prone to errors and artifacts with the sensitive assays (interference between coloured extracts and some identification techniques, for instance), or be unfruitful due to the high number of present compounds or the low titers. Fractionation steps can be helpful at this step but they also increase the number of samples and tests to perform [152]. Thirdly, at the purification and identification steps, it is important to avoid

redundancy if some already known metabolites are present. Such "dereplication" processes are seen as a sizeable drawback compared to the chemical synthesis route that handles well-defined compounds. A final example of the scientific challenge of natural products discovery relates to their structural complexity that renders medicinal chemistry for pharmacological optimisation quite tricky. As structural complexity is not an absolute obstacle and even provides advantages over the likeness of compounds in chemical libraries, one of its major drawbacks lies in the synthesis and scale-up processes that can require complex and costly engineering steps.

Fully synthetic approach

At the other end of the drug discovery spectrum, the modern view of the "magic bullet" approach uses medicinal chemistry and rational strategies to produce pure compounds with therapeutic specificity and utility. While the sulfonamide drugs in the 1930s were the first antibacterial representants of this strategy, it has led some of the newest therapeutic classes in use today, such as the oxazolidinones (introduced in the clinic in 2000), an entirely synthetic family with the only representant being linezolid.

This synthetic approach draws on the (general) basic principles of drug discovery: identifying a target, finding lead compounds through screening, optimising their druggability and testing their pharmacodynamics and proof of concept (Figure 28). These steps are quite general, with shared techniques across all therapeutic areas, and only where elements have a particular relevance to antibiotics are they commented in this paragraph.

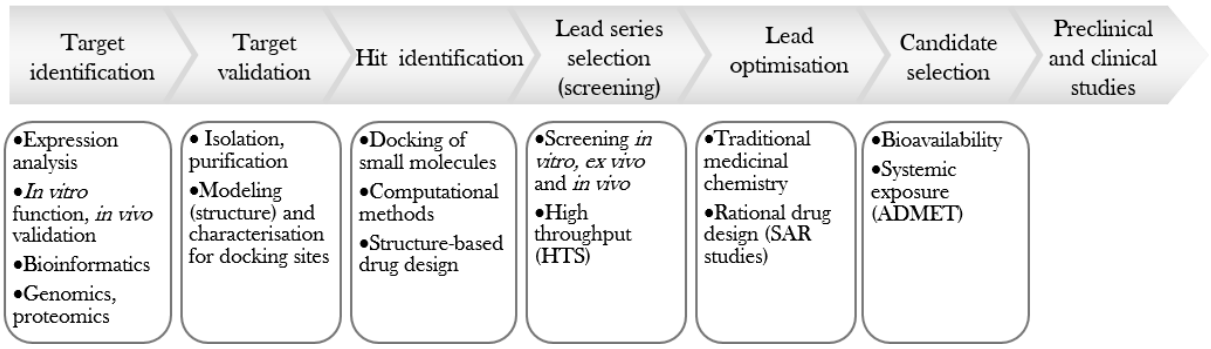


Figure 28. Steps in synthetic drug discovery; SAR: Structure-Activity Relationships. Adapted from [153] and [154].

When it comes to antibiotics, the synthetic pathway was off to a very promising start (and the research of natural products met with a near-demise) when the enthusiasm for genomics and other molecular-based techniques tried to take over the drug discovery field as a whole in the 1990s [155]. The first potential application was the discovery of new targets. In 1994, SmithKline Beecham started a bacterial genome sequencing project on two main Gram(+) pathogens, *S. aureus* and *S. pneumoniae*, and the first genome sequence to be completed was that of *H. influenzae* in 1995 [73].

The interest was that completed bacterial genome sequences reference all possible molecular targets, whether essential determinants of the cell cycle and bacterial viability, or pathogenicity and virulence determinants, but the challenge was to identify them.

In addition to applications for bacterial targets, the major impact and second application of genomics in antimicrobial drug discovery has been in the area of medicinal chemistry and antibiotic drugs. Yet what was seen as a great source of innovation with molecules acting by totally novel mechanisms has not delivered the expected results, and one explanation relates to the historical bias towards previously examined receptor and enzyme targets, lowering the odds of finding agents acting on novel pathways. For example, during the genomic frenzy, the screening programme that Abbott developed to target the bacterial topoisomerase I produced few leads, none of which turned into a drug candidate [156]. Another idea presented as a potential (if not unconventional) lead discovery approach was to isolate the DNA from hard-to-cultivate species and insert it into expressing bacteria, such as *E. coli* [157]; a screening of the produced metabolites would then identify possible antibiotics. So far, this approach has not led any result, despite a demonstrated proof of concept, and the interest wound down around 2005. But the impact of bacterial genomics on antibiotic discovery could be immense, whether in discovering new classes of drugs or even new therapeutic strategies against pathogens. The challenge would be to bridge the gap between the theory of a metagenomic library and the industrial scale, yet with the majority of companies working on this approach having re-focused their efforts, the method is unlikely to deliver the next breakthrough antibiotic.

Another approach at the target identification level was to make use of the proteomics capabilities and the expanding knowledge on bacteria's antibiotic biosynthetic pathways to reprogram the assembly lines (NRPs, PKSs – see point (d) below) to create a library of natural products, yet maybe because of the lack of quick results combined with a global industrial divestment of the field, this approach has been dropped. As a result, the optimism coming from these potential new applications, whether in finding new targets, optimising old ones, or uncovering new drug leads, has wound down with the results of their bad performances : no new drug candidate originated from these disrupting technologies. For instance, the application of techniques such as high-throughput screening (or HTS, with recent examples of this approach reviewed in [152]) was not as successful in identifying new targets as it was originally thought. An analysis of the failures of this approach, highlighting the scientific challenges of this second drug discovery method, revealed that the lack of a strong antibacterial activity *in vivo* was among the main reasons for the dismissals of the chemical libraries' compounds, due to either poor penetration properties or active efflux, properties that are very difficult to correct by medicinal chemistry.

It is possible that the lack of results with the HTS approach, the automated way to discover drug leads in the 21st century, may have deterred companies to pursue their efforts in the antibiotic field, preventing them from putting additional investments and exploring other drug discovery routes altogether. The chemical route having failed to accelerate the antibiotic discovery process, many companies saw the natural route, rather unattractive, as the only remaining option. Natural drug discovery may be seen as a thing of the past as well as a tedious process, yet it is still a very promising source of future breakthrough antibacterial drugs, thanks to the unparalleled structural diversity of the products, their production as a culmination of millions of years of evolution and the untapped, large potential in species that have not yet been exploited.

d. Types of antibacterial products

Nature versus man's chemistry: the essential duality

After the description of the possible approaches and routes to antibiotic production, this section turns to the evaluation of the output: the types of antibiotics that have been successfully developed. This evaluation starts with a striking observation. While Domagk's fully chemical dye, Prontosil, was used in 1935 in infected mice against *Streptococcus* and led later on the sulfamides family to be introduced in the clinic, Dubos' natural gramicidin was extracted from the soil bacterium *Bacillus brevis* in 1939 and used locally against infections. While the natural cephalosporin C was isolated from the fungus *Cephalosporium acremonium* in 1948, a vast programme of chemical enhancement around the common β-lactam core led to the creation of the most populated family of antibiotics, the cephalosporins. Man's versus the microorganism's chemistry: this is the duality that characterises the antibiotic class so well.

Antibiotics as natural products

Prokaryotes embody the most prolific group of antimicrobial producers. What has turned them into masters of the antibiotic? What mankind has classified into 'antibiotics' are actually metabolic compounds, secondary metabolites only produced in the stationary phase of the bacterial cycle. They have indeed no role in the sense of traditional growth, but they are released when bacteria face competition for space or nutrients. The antibiotic compounds then take on a variety of roles, from external communication between colonies (some have been compared to bacterial hormones) to lethal weapons in a more aggressive fight for survival. Antibiotic-producing genes are activated after external signals, serving a function known as quorum sensing, are read by the bacterium. Following a typical cascade do these signals lead to an internal activation of many regulation pathways, up to the genetic level with derepression or up-regulation of specific clusters.

These pathways have existed for billions of years in the biosphere [158]. In addition to when the antibiotics are produced, the knowledge of how they are produced is useful for a natural screening

method to be productive. The main element about antibiotic synthesis is that, since the molecules are rarely (poly)peptides, they do not employ the traditional synthetic process of ribosomal transcription. Rather, for the peptidic antibiotics, a large proportion is assembled on very large protein templates by non-ribosomal peptide synthetases (NRPSs), allowing the polymerisation of monomers in an assembly-line-like mechanism and employing non-proteogenic amino acids [159]. This NRP way includes precursors to β-lactams (except the carbapenem skeleton), gramicidin, bacitracin or lipopeptides. Another synthetic pathway for aromatic polyketide-types antibiotics employs polyketide synthases (PKSs) with similarities to the synthesis of fatty acids (succession of cycles including initiation, a various number of elongation steps, and termination) [160]; this pathway is used for the four rings of the tetracycline family or erythromycin. Finally, there exist other antibiotic assembly lines, such as hybrids between NRPs and PKSs for pristinamycin or rifampicin.

But these metabolites display extensive multifunctionality, and limiting them to killing functions against surrounding microorganisms would be a limited anthropomorphic view: antibiosis is just one of the many activities of the bioactive small molecules. They can be involved in cell-cell signalling [161] or additional metabolic functions (for example, penicillin is employed in cell wall turnover [162]). The need for compounds displaying lethal properties against other microorganisms is justified by the bacterial physiology: unlike more evolved organisms, bacteria cannot count on an overarching immune system that would neatly coordinate an army of various defensive tools to organise the fight against invading species.

The production of antibiotics is not an isolated one and comes most of the time with the expression of the associated counterdefense mechanism. If the bacterium produces an antibiotic that could act against itself as well (if it possesses the targeted biomolecule, exposed to the antibiotic action), it is very likely that the producing strain will also produce a counter-mechanism, the antidote to its own poison. Antibiotic synthetic pathways and resistance pathways have co-evolved such that genes encoding resistance mechanisms (pumps, enzymes...) are usually found within the transcriptional clusters of the very antibiotic genes [111]. Still in need for auto-immunity but in a different strategy, some bacterial producers will synthesise an inactive form of the antibiotic whose activation is dependent on its export (for instance, activated with extracellular enzymes: oleandomycin with a secreted glycosidase [163]). The self-protection mechanisms can give insights into and predict the resistance mechanisms likely to be developed by other strains: for instance, to protect itself against their erythromycin, *Streptomyces* species have a constitutionally-modified target (the ribosomal adenine necessary for drug binding, A_{2058}), which is also methylated in bacteria with acquired resistance to erythromycin (through the passing of the methyltransferase resistance gene, *ermE*).

Antibiotics as artificial products

Since the development of a couple of fully synthetic antibiotic classes, oxazolidinones by DuPont workers [164], and fluoroquinolones – although this class can also be classified under the hemisynthetic compounds, as nalidixic acid, the core structure they are based on, originated from a natural source –[20], man has not outwitted nature at designing new antibiotic scaffolds. This does not prevent a lot of creativity to enliven discussions around the next-generation antibiotics. Anything that is druggable (or not even so) has been thought of to become the next antibiotic breakthrough or to embody the evolving scientific paradigm in the combat against pathogenic bacteria, and in the context of a drying pipeline calling for novel drugs, very different and original leads have been put forward.

Exploiting innate immunity with antibacterial compounds such as defensins, cecropin or magainin [165], inhibiting the bacterial adhesion to the host's mucous membranes or eliminating resistance genes [166] are among the contemplated approaches. Researchers also had a try at finding applications for gene therapy in infectious diseases, having identified promising targets such as efflux pumps in Gram(-) species, where gene knockouts would not only restore susceptibility to a wide range of antibiotics, but would also decrease the bacterial virulence [167]. Maybe with the democratisation of gene therapy in a not-so-distant future would this approach realise its full potential, eased by the absence of a nucleus membrane in the bacterial cell. Such an idea of democratisation and lowered costs with more widespread and common use could also benefit monoclonal antibodies in bacterial infections, a new scientific paradigm as well, put forward and developed by Arsanis Biosciences [168], [169] or Aridis Pharmaceuticals [170], [171]. The future of antibacterial drug discovery may also be examined in the light of vaccines: whether they have a true potential in this field would be answered by the capacity of overcoming the replacement problem (bacterial species escaping the educated immune system by mutating their surface proteins, an issue currently encountered with *S. pneumoniae* and its vaccines [172]). Another parallel with virology, the disarming therapeutic approach would consist in eliminating the infection and virulence capacities of a bacterium, rather than killing it completely; because of the much smaller life threat that this therapeutic option would represent to bacteria, it has been hypothesised that it will generate much weaker selection for resistance, but this approach is very much in its infancy [173], [174]. Because virology and bacteriology seem to interact a lot, the potential outcomes of this collaboration also include the use of viruses as antibacterial agents. Although this idea of therapeutic bacteriophages is not new (it has been started by Felix d'Herelle in the 1920s and, although the studies with bacteriophages were not actively pursued in the US or in Europe, phages

[20] Bedaquiline is also a synthetic antibiotic, yet its exclusive use against *M. tuberculosis* excludes it from this discussion.

continued to be used in Russia and Eastern Europe [175]), it is periodically rekindled, despite very effective resistance mechanisms conferred by mutations.

B. PAST, PRESENT AND (SOME) FUTURE PROSPECTS IN ANTIBIOTIC DRUG DISCOVERY

Most of the current antibiotics have been discovered in the 20th century, but no new therapeutic classes have been introduced since the 1980s (Figure 29). The methods for antibiotics discovery discussed above, such as high-throughput screening, have not resulted in many commercially viable results [176]. Additionally, over the 1980-2009 period, 43% of approved antibiotics were withdrawn because of safety reasons or commercial reasons (lack of market). This rate is much higher than for non-antibiotic drugs, where it amounts to 13% [177]. In addition to being of uncertain security once launched, antibiotics are difficult to discover: "the low-hanging fruit has been picked", according to Spellberg's formulation, and this image has been used to justify the discovery void in the evolution of antibiotic discovery [178].

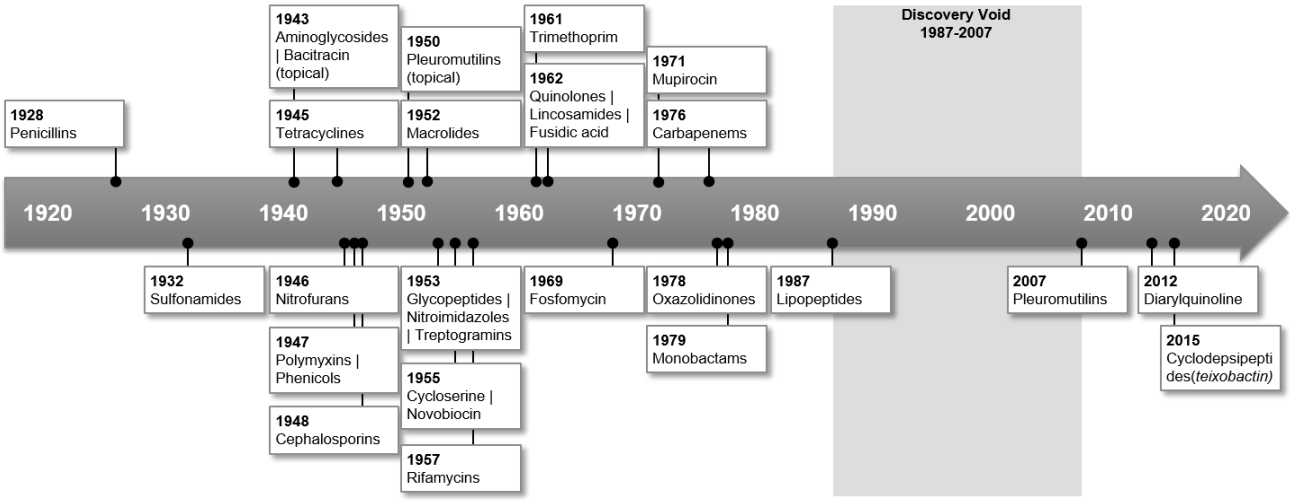


Figure 29. Timeline of discovery of classes of antibiotics. Adapted from [179].

When it comes to the most recently approved drugs and the current pipeline, there is a striking imbalance between classes of antibiotics with regards to their bacterial target. Because of their outer membrane and their various porines, Gram(-) bacteria are much more difficult to target and eradicate: in addition to the potential antibacterial compound, drug discovery must also tackle two key points, namely entry and efflux. These extra challenges are reflected in the imbalance in terms of marketed and developed antibiotics between the two Gram categories (Table 3).

As of March 2016, about 37 antibiotics were undergoing clinical evaluations in the US [180]. Analysing the pipeline of antibiotics from phase I to phase III, 30% to 40%[21] are predicted to have an activity against Gram(-) pathogens. It is true that the difficulty of reaching the target because of the outer membrane is a specific challenge, but it is paired with a special opportunity in terms of market: because of the concerning growing pan-resistance, Gram(-) infections represent a bigger commercial opportunity. The market for Gram(-) infections is estimated at $4bn, twice the size of the largest market opportunity within Gram(+) infections, MRSA (methicillin-resistant *Staphylococcus aureus*), a $2bn market [181].

	Recently Marketed(1)		**In Late-Stage Development(2)**	
Gram(+)	linezolid	ZYVOX (Pfizer)	ceftobiprole*	(Basilea)
	daptomycin	CUBICIN (Merck)	solithromycin	(Cempra)
	tigecycline	TYGACIL (Pfizer)	zabofloxacin	(Dong Wha)
	telavancin	VIBATIV (Theravance)	lefamulin	(Nabriva)
	ceftaroline	TEFLARO (Actavis)	delafloxacin	(Melinta)
	dalbavancin	DALVANCE (Actavis)	cadazolid	(Actelion)
	oritavancin	ORBACTIV (TMC)	tedizolid	(Merck)
	telizolid	SIVEXTRO (Merck)	iclaprim	(Motif Bio)
Gram(-)	ceftolozane/tazobactam	ZERBAXA (Merck)	eravacycline	(Tetraphase)
	ceftazidime/avibactam	AVYCAZ (Actavis/AZ)	plazomicin	(Achaogen)
			meropenem+ vaborbactam	(TMC)
			relebactam	(Merck)
			S-649266	(Shionogi)
			omadacycline	(Paratek)

*Table 3. Marketed and developed Gram(+) and Gram(-) antibiotics. *: approved in Europe. (1): ranked by chronological year of approval; (2): as per status in the US market, includes only drugs having reached Phase 3 trials; (3): originator: VANCOCIN (Shire). TMC: The Medicines Company. AZ: Astra-Zeneca. Note: selection of recent molecules only. Data as of March 2016. Adapted from [180], [181].*

Of concern is the fact that, in both Gram(-) and Gram(+) categories, there is no new chemical family. Without significantly different chemical structures, the novel "me-too" antibiotics may experience resistance very quickly with bacteria that were selectively pressured under similar previous drugs. Indeed, and illustrated by an analysis undertaken by the Review on antimicrobial resistance [182], the bulk of antibiotics in development are neither breakthroughs nor compounds that are the most

[21] Depending on the inclusion or not of the candidates whose action on Gram(-) bacteria is only deemed 'possible' at this stage.

clinically useful (Figure 30), and about a third (16 out of 41) of these drugs in development display activity against bacteria that represent the most pressing needs, namely multidrug resistant Gram(-) strains. As outlined above, antibiotics against Gram(-) bacteria are particularly sought-after, whereas the range of drugs to combat most of the Gram(+) bacteria is already robust. The 'high priority' category gathers drugs that may be active against Gram(-) pathogens, and the 'medium priority' one, drugs that are expected to show activity against a CDC urgent threat pathogen. The rest is classified as 'low priority'. It is striking to note the low investment in terms of drugs for high-priority pathogens: market realities, rather than health needs, determine R&D priorities, as described in Part 3.

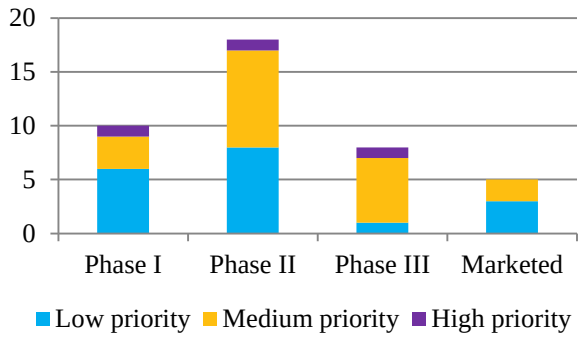


Figure 30. Antibiotics in development or approved broken down by priority of need. Pipeline data as of December 2014. From: [180], [182].

Finally, to briefly note a few trends in the near future of antibiotic therapy, the methods that have the best potential to effectively deliver novel agents, among the ones cited in (d), could well be represented by non-culturable bacteria (NovoBiotic Pharmaceuticals), bacteriophages (AmpliPhi Biosciences, GangaGen Biotechnologies, Phage Biotech) [183] as well as parallel approaches to prevent the side effects of antibiotics on the gut flora (Da Volterra). Biologics may as well come sooner than expected in the antibacterial therapeutic arsenal, as the FDA is also about to discuss the biologics license application (BLA) of bezlotoxumab in June 2016, for the indication of prevention of *C. difficile* infection recurrence (Merck) [184]. Whatever direction is taken, the common traits and striking feature surrounding the future of scientific discovery of antimicrobial agents, their grand innovation and groundbreaking concepts, impassionate the many discussions on the topic with an exciting view of what lies ahed [185], [186], [187].

SECTION 2. SOIL BACTERIA AND UNCULTIVABLE MICROORGANISMS

A lesson drawn from the previous section is that, when comparing the two drug discovery routes (natural and synthetic), the synthetic one, chiefly represented by the oxazolidinones, has been quite

unable to replace natural products, which are therefore the most logical approach for new compounds. Yet how can the weak industrial interest in natural antibiotic research be revived? Outweighing the scientific challenges may be the opportunities that arise from the fact that less than 0.5% of the estimated 2–3 billion microbial species have been identified [58], and about 99% of all species in external environments are uncultured so far [188][22]; needless to say that this represents an incalculable number of potential antibiotics[23]. These yet-to-be-identified, antibiotic-producing species have established themselves in various environments, from the most extremes, as in the Arctic oceanic trenches [189], to the ones closest to us, but yet unexploited. Among all these exciting novel sources of antibiotics, this thesis will only focus on soil reservoirs – and their untapped variety of useful bacteria that recently brought us teixobactin.

A. THE HIGH POTENTIAL OF SOIL

A brief ecology of soil

Soil is best defined as the interface between the lithosphere, which provides minerals, and the biosphere, which provides organic compounds. As a brief introduction, its main role is to support the life of plants, animals, and an abundant microflora: it is estimated that 1g of soil can gather more than 10^9 bacteria, represented by a diversity of between 4,000 and 7,000 genomes [190].

This bacterial biodiversity represents the greatest guarantee for the stability and richness of the soil. Indeed, each species has a specific role and function but colonisation depends on the features of the soil. The first function of bacteria in soil, the structuring of the soil, results from a principle of reciprocity: if the soil displays attractive characteristics for bacteria to colonise it, they will do so, and will in return alter the parent rock and stabilise the aggregates. Poorly ventilated soils, or soils with low water holding capacity (sandy) are less favourable for bacterial colonisation and survival, and as a result, their aggregates are less stable, resulting in the cementation of the sand. Therefore, the composition of the soil combined with bacterial growth determines the friability, water infiltration and retention rates, ventilation properties and erodibility of the soil [191]. A second role of soil bacteria is the supply of nutrients to plants, for example, nitrogenous compounds that they can metabolise. In a similar fashion, they participate in the degradation of xenobiotics through their versatile metabolic activities, and they also contribute to controlling soil-borne plant diseases to some extent (bacteria compete against fungi for colonisation).

When it comes to their ecology, bacteria are mostly distributed in soil micropores (>80%), because of their bigger concentration of cations and organic matter. Such structures also diminish their

[22] They are unable to grow under the current laboratory conditions.
[23] Actually, some bold researchers have estimated the number of antibiotics still to be discovered (from actinomycetes alone) well above 10^5 [349].

exposure to protozoans, nematodes, and larger animals. Also, when bacteria settle in, as described above, they will modify the local chemistry, creating an ecological niche with variations in pH, oxygen rates, and nutrients.

A fruitful source of antibiotics

Soil has always been a go-to source for new antibiotics. At the very beginning of the antibiotic discovery had Rene Dubos unearthed his gramicidin in 1939 from *Bacillus brevis* [192], followed by Waksman's team a few years later: they successfully turned to the soil bacteria, initially *Streptomyces griseus*, and with streptomycin began the well-stocked family of antibiotics of *Actinomycetes* origin. The soil-based *Streptomyces* family has demonstrated genius for antibiotic production (Table 4), yet expanding an already profuse family is still feasible by developing new ways of growing previously uncultivable species, which should hopefully lead to even more impressive drug discovery results.

Species	**Antibiotic**
Streptomyces noursei	nystatin
S. nodosus	amphotericin B
S. erythreus	erythromycin
S. fradiae	neomycin
S. griseus	streptomycin
S. rimosus	tetracycline
S. orientalis	vancomycin
S. mediterranei	rifamicin
S. venezuelae	chloramphenicol
S. alboniger	puromycin

Table 4. Antibiotics extracted from Streptomyces *soil species.*

B. NEXT-GENERATION MICROBIOLOGY CULTIVATION AND SCREENING SYSTEMS

Since the overwhelming majority of bacterial species, including soil bacteria, are unable to be cultured in the laboratory, unleashing the drug discovery potential of such an untapped resource will depend on innovation in cultivation methods. With the limits of *in vitro* cultures with Petri dishes being met, a shift in the culture paradigm is being initiated and the strategy is to mimic the environment of the target organisms. *In situ* growth has been tried since the early 2000s and proven successful in cultivating demanding microbial species: with hollow-fiber membrane chambers, substrate membrane systems or diffusion chambers can one use the naturally occurring compounds present in soil to cater for the specific requirements of bacteria and grow isolated colonies in

microchambers [193], [194]. However, many methods were poor substrates for automation, remaining tedious, technologically complex and time-consuming processes.

The next-generation cultivation systems that will manage to streamline the entire cultivation workflow, with high-throughput platforms, increased hit rates, faster cycle times and lower costs, represent a promising developing area. For instance, gel encapsulation within permeable macrodroplets, a device that encapsulates individual bacterial cells and allows them to form pure colonies when bathed in their environmental media, has been used for actinomycete cultivation at Cubist Pharmaceuticals (now part of Merck & Co.) [195]; similarly, but using microdroplets, then flow cytometry to separate the droplets, millions of microfermentations can be performed for previously uncultured bacteria [196].

The iChip technique

Another example, most recently developed and particularly relevant for the culture of soil bacteria, is the isolation chip "iChip" technology [197]. Here is how it works: a sample of soil is taken, diluted and incubated with an iChip plate, which consists of hundreds of small holes, such that one bacterial cell is delivered to a given channel. The chip is then covered with semi-permeable membranes on both sides and placed back into the soil sample (Figure 31).

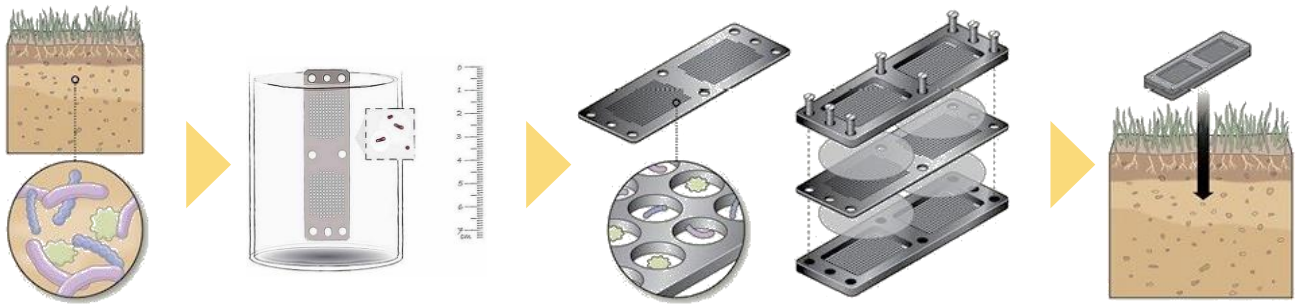


Figure 31. The iChip technology. A sample of soil containing the bacteria of interest is suspended in liquid agar; the agar, then solidified, immobilises one cell per through-hole in the iChip; added to the central plate are two other, flat hydrophobic polycarbonate membranes assembled to compose a system of miniature diffusion chambers; the iChip is then returned to the environment for incubation. Adapted from Granberg [198] and [199].

Compared to traditional technologies, the number of cells forming colonies in iChips is substantially higher (c.50%) than that in standard petri dishes (1%) and at least as good as that of the previous methods of diffusion chambers it has evolved from. Advantages over the diffusion chamber include the ease of iChip operation (assembly takes less than five minutes), the simplicity of growth scoring and removal and, relevant to scale-up processes, the fact that the plate can be replaced by a

microtiter plate, allowing for high-throughput applications. Then, once a colony is formed by cultivation in an iChip, it can be grown *in vitro* by subculturing on standard media ("domestication") and scaled up. It is estimated that with the current success rates of cultivation and domestication, a researcher can produce about 100 pure cultures of novel bacterial species per day [197]. Such a massive parallel *in situ* cultivation may move the limiting factor of natural antibiotic discovery from cultivation to downstream analyses (such as identification of leads or purification).

Other techniques

It is also worthy to note that, apart from innovation in cultivation methods, solutions to unleash the potential of the uncultivated and unidentified bacterial species can also take the form of other discovery methods, as briefly outlined in the synthetic drug discovery pathway (see Section 1 A c), such as with next-generation gene sequencing, allowing scientists to screen collections of DNA from the soil microbiome ('genome mining'). Such innovative techniques are eliminating the need for cultivating the demanding species themselves (with the cloning of metagenomic DNA in vectors) or eliminating the need for cultivation altogether ('genome mining' assisted by bioinformatics). Advances in strain dereplication based on 16S rRNA sequencing to achieve a finer level of strain discrimination and ultimately optimise results in lead compounds identification are also significant improvements in the natural discovery process, together with developments in chemical identification (increased sensitivity of HRMS, more accurate tools such as Fourier transform ion-cyclotron resonance mass spectrometry, or FTICR-MS) to improve the chances of finding new molecules even when produced in very small quantities [195].

Coupled with the substantial amount of uncultured bacteria, these techniques are as many encouraging advancements and reasons to be cheerful for the outlook of natural antibiotic production. A prototype version of the iChip, the diffusion chamber technology has already successfully isolated promising compounds from soil species, such as neocitreamicin (*Nocardia* spp) and Novo10 (*Oerskovia paurometabola*) [199].

SECTION 3. TEIXOBACTIN

A. THE UNEARTHING OF AN INNOVATIVE MOLECULAR ENTITY

All the above techniques are not mere future concepts or ideas, they have tangible results that can vouch for their practical applications. Illustrating the success of the iChip technique is the identification of teixobactin [200]. When about 10,000 soil bacterial strains were isolated from the iChip plate and their extracts screened for activity against *S. aureus*, a product from the

provisionally-named *Eleftheria terrae*, a Gram(-), previously unidentified β-proteobacteria from a new genus (related to *Aquabacteria*) showed good activity and was purified and identified (Figure 32). This molecule represents a dual interest in the context of this thesis: not only does it epitomise the innovation in scientific practices (in terms of culture and identification of producing species) that can provide answers to the scientific challenges that antibiotic development faces, but it also constitutes the first member of a new family of antibiotic, and a new vein to fuel an antimicrobial discovery pipeline lacking true novelty is welcome (see Section 1 B).

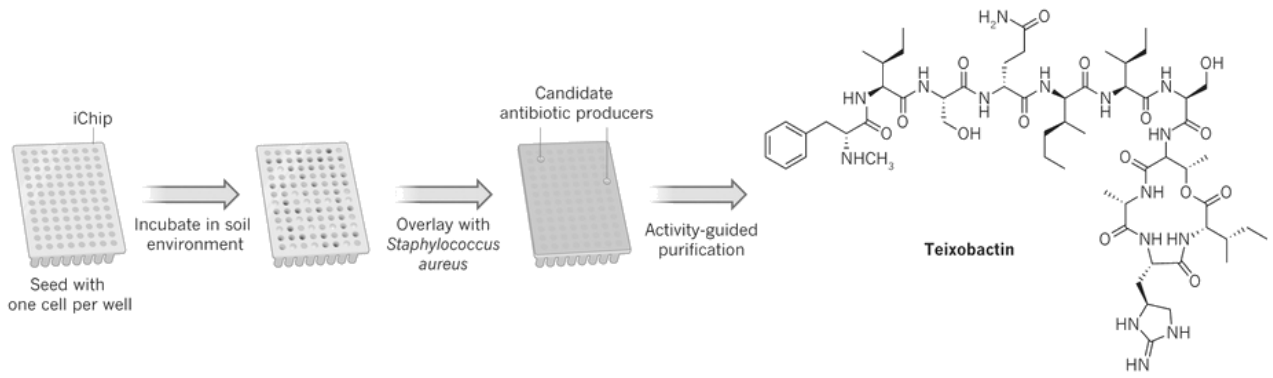


Figure 32. Identification of teixobactin with the iChip technique. From [201].

B. INTRODUCING TEIXOBACTIN

a. Structure

This molecule is a polypeptide, specifically a cyclodepsipeptide containing unusual amino acids among its eleven components: N-Met-phenylalanine, isoleucine, serine, glutamine, two isoleucines, another serine, and a cyclised (lactone) association of threonine-isoleucine-enduracididine-alanine. Synthesised under the NRPS pathway, teixobactin is derived from two genes, *txo1* and *txo2*.

b. Mechanism of action and pharmacokinetics

Teixobactin is a novel inhibitor of peptidoglycan synthesis that acts by binding to cell wall precursors. Due to its amphipathic nature and the positively-charged amino acids (in the form of ammonium salts), teixobactin specifically binds to the near-membrane, phosphosidic parts of cell wall and teichoic acid precursors, lipid II and III[24] (Figure 33). As a brief reminder, the biosynthetic pathway of the cell wall requires several steps (see Appendix 3, Insert 1). The initial building block,

[24] Teixobactin also binds to lipid I *in vitro*¸but this precursor is not flipped to the other side of the cytoplasmic membrane, making it inaccessible by the antibiotic *in vivo.* Similarly, teixobactin binds to undecaprenyl-pyrophosphate.

N-acetylmuramic acid-pentapeptide (MurNAc-pentapeptide) is produced in the cytoplasm before being coupled with a membrane carrier (undecaprenyl-phosphate). The resulting membrane-anchored compound is called lipid I. The addition onto the MurNAc moiety of a second osamine, GlcNAc, results in the formation of lipid II, which is further modified before being flipped to the other side of the cytoplasmic membrane, where it is targeted by teixobactin. A parallel pathway, but involved in the biosynthesis of teichoic acid, leads to the formation of lipid III, by the addition of a GlcNAc on the undecaprenyl-pyrophosphate membrane lipid; some additional steps are undertaken before flipping in onto the extracellular environment, making it also accessible to the drug. This double impact prevents the formation of a functional cell envelope. This mechanism is indeed new, because teixobactin does not bind to the enzymes involved in the synthesis of the cell wall, unlike the wide family of β-lactams, nor does it bind to the pentapeptide component of it, unlike the glycopeptide family (see Appendix 3).

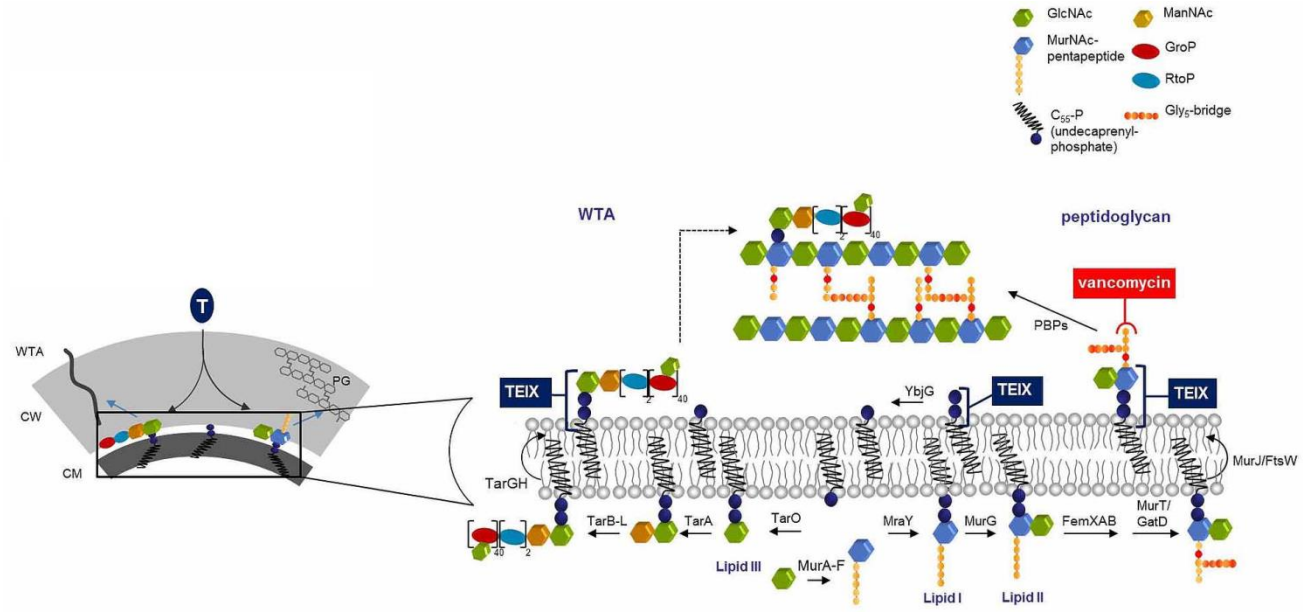


Figure 33. Mechanism of action of teixobactin. Teixobactin acts in the extracellular media, at the cell wall's level, by binding to precursors: lipid II and III when flipped over, inhibiting both the cell wall biosynthesis and wall teichoic acid biosynthesis pathways. Vancomycin's binding target is shown for comparison. CW= cell wall; CM= cytoplasmic membrane; TEIX= teixobactin; WTA= wall teichoic acid. From [200].

Teixobactin's pharmacokinetic parameters are detailed in Appendix 6 (Figure 5); the molecule displays a favourable profile, with a serum concentration above MIC maintained for four hours. Tests on mammalian cells showed no *in vitro* toxicity, no haemolytic activity as well as no genotoxicity. In addition to its remaining stability and potency in the presence of serum, the *in vivo* toxicity was low (this is expected since the membrane-disrupting activity of teixobactin is dependent upon its binding to lipid II or III, specifically found in bacterial cells). When it comes to its *in vivo* pharmacodynamics (only the animal models at this stage), the efficacy of teixobactin was

significant in a mouse septicemia model with MRSA, a thigh infection model with *S. aureus* and an infection with *S. pneumoniae* (detailed results in [200]).

c. Spectrum of activity

Teixobactin is active against most Gram(+) species, including difficult-to-treat enterococci and resistant strains, *B. anthracis* and *M. tuberculosis*; it has no activity against Gram(-) species (Table 5).

Species	**Teixobactin MIC_{90} (µg/mL)**
S. aureus (MSSA)	0.25
S. aureus (MRSA)	0.25
S. aureus (VISA)	0.5
E. faecalis (VRE)	0.5
E. faceium (VRE)	0.5
S. pneumoniae (peni-R)	<0.03
S. pyogenes	0.06
B. anthracis	<0.06
C. difficile	0.005
P. acnes	0.08
M. tuberculosis H37Rv	0.125
E. coli	25
P. aeruginosa	>32

Table 5. Spectrum of activity of teixobactin against selected bacterial species. Peni-R= penicillin-resistant. From [200].

Of particular interest is its excellent activity against *Staphylococcus aureus*, including resistant strains, which may represent a welcomed therapeutic alternative for these burdensome health threats (see Part 1). *S. aureus*, a Gram(+) microorganism that is naturally present in the bacterial flora of healthy humans, has successfully developed counter-attack mechanisms to several of the antimicrobial weapons targeted against it; they are briefly reviewed here to understand the comparators used for teixobactin. Soon after one of the first antibiotics, penicillin G, became clinically available in the 1940s, *S. aureus* started producing a penicillinase. This enzyme shortly disseminated among all the clinical strains of the species, together with a phenotype of multidrug resistance that also excluded the usage of tetracycline, streptomycin, and erythromycin as antistaphylococcal agents. The successful development of another β-lactam compound, methicillin, as well as agents in the cephem family in the 1960s, brought some solutions whose temporality was then evidenced by the emergence of methicillin- (and cephem-) resistant *S. aureus* (MRSA). Initially confined in the hospital setting of nosocomial infections, MRSA has nowadays diffused in the community, threatening to progressively replace the methicillin-susceptible strains (MSSA) and

leaving only a few drugs effective to treat the various infections it is responsible for. Vancomycin is one of them. Unlike methicillin, vancomycin is not a drug that was purposely developed to resist MRSA, but is instead a relatively old molecule for which medical interest was revived at the dawn of *S. aureus*'s resistance emergence. Yet the bacteria started to gradually resist this antibiotic as well, putting the emphasis on the urgent need for anti-VISA and anti-VRSA compounds: for these resistant strains, few therapeutic options, such as daptomycin or linezolid, remain.

Interestingly, teixobactin was found superior to vancomycin in killing late exponential phase populations (Figure 34). Also, in addition to a potent activity against MRSA and VISA strains (Table 5), teixobactin displays an MIC range of 0.12 – 0.5 μg/mL for daptomycin-non-susceptible strains, and of 0.12 – 0.5 μg/mL too for linezolid-resistant strains, which predicts a potential usefulness as a last-line therapeutic agent. Teixobactin is able to counter the resistance mechanism of VISA/VRSA strains, because their escaping of vancomycin is based on the modification of the proteic part of the peptidoglycan building block, which is not where teixobactin binds.

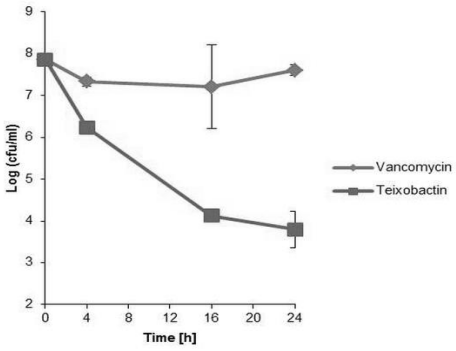


Figure 34. Activity of teixobactin compared to vancomycin on strains grown to late exponential phase and challenged with vancomycin (blue) or teixobactin (red). From [200].

C. A NEWCOMER WITH NO RESISTANCE?

In their seminal article on the discovery of teixobactin [200], the authors reported a notable absence of resistance, which was induced experimentally by plating *S. aureus* and *M. tuberculosis* strains on plates with low doses (four times MICs) of teixobactin over a period of 27 days: no resistant mutants appeared.

According to the authors, this absence of (immediate) resistance under laboratory conditions was explained by the fact that the target is not a protein and hence, what is considered the quickest and easiest path to resistance, the modification of the proteic target by genetic mutations of phenotypic impact, is ruled out for teixobactin. Since its target is lipidic, it is alleged to have fewer alteration possibilities. Indeed, with different biosyntheses, lipids are produced from organic precursors and

gene-encoded enzymes, whereas the assembly of peptides is guided by a translated ribonucleic structure, which hence directly reverberates any mutation in the coding sequence. However, this explanation is not vouching for a complete lack of resistance, which, again, is a natural and unavoidable mechanism that bacteria develop to survive.

Resistance is a problem that must be dealt with head on, and academia and research can contribute much to the discussion. It has been the case that some innovative antibiotic classes had failed to be viable because of the overwhelming resistance mechanisms that had developed quickly (examples include inhibitors of peptide deformylase [202] or LpxC inhibitors [203]). While the mechanisms for teixobactin have not been experimentally unveiled yet, with a good understanding of the development of resistance supported by examples can one hypothesise about the possible ways resistance to this novel entity is likely to emerge. Figure 35 provides a framework for organising this reflection.

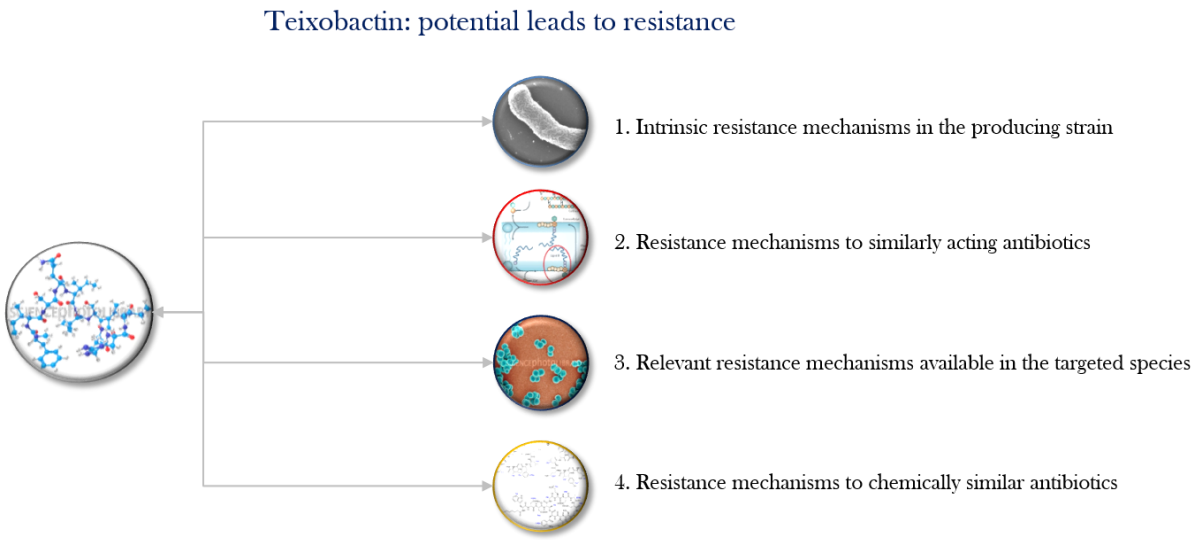


Figure 35. Possible ways resistance to teixobactin can develop: borrowing the resistance mechanisms developed against other agents targeting the same pathway, fall prey to resistance mechanisms developed at a broader level by the bacterial strain, or leverage specific mechanisms for similar chemicals.

Intrinsic resistance mechanisms from the producing strain

How could resistance to teixobactin emerge? A first idea to discuss the apparition of resistance is to analyse the protection mechanisms of the producing strain against its own killing compound. *E. terrae* is a Gram(-) bacterium that protects itself against teixobactin by exporting it across its outer membrane, a sizeable permeability barrier that prevents it from re-entering and altering the cell wall. This is a classic, non-specific resistance mechanism and since the target bacteria (Gram(+)

bacteria) cannot display an outer membrane, this intrinsic resistance is not telling about a future resistance mechanism that could be transmitted through inter-species genetic exchange.

Resistance mechanisms to similarly acting antibiotics

A second option to explore is the appearance of resistance in antibiotic families and compounds that have a comparable target or mechanism of action. Antibiotics that bind to the lipid II cell wall precursor include glycopeptides, lantibiotics, and defensins. Resistance is likely to eventually emerge from horizontal gene transfer with a neighbouring soil bacterium, as this has been the case for many of the previous cases of resistance transmission, but, to make an educated guess about the nature of the resistance mechanism, it has to be noted that teixobactin's target, the pyrophosphate-sugar moiety of the lipidic precursors, is highly conserved among bacteria. This implies that target modifications, or even the bypass of this element in the biosynthesis process, is unlikely to be a viable resistance mechanism. Indeed, lipid II has an essential role and modifications that would alter the precursor while retaining its physiological function have never been described. Additionally, quantitative target changes through level variations have no impact either on the activity of nisin, a lantibiotic compound [204], so are likely to not affect teixobactin either. This leaves resistance options such as an antibiotic-modifying enzyme.

Therefore, among antibiotics having the same target, is enzymatic modification a frequent resistance mechanism? The resistance mechanisms to the first category, glycopeptides, have already been described in B (c) and, since they affect the peptidic part of the target and not the lipidic part, they cannot be applied to teixobactin. While the third category, defensins, does not gather many therapeutic compounds, the second category, lantibiotics, is interesting to discuss: lantibiotics, such as nisin, mersacidin or epidermin do not only act by binding to the pyrophosphate moiety of lipid II[25], they are also closer in structure to teixobactin than the other cited categories, as they are peptide antibiotics, characterised by intramolecular rings between amino acids, and by elongated, cationic and amphiphilic structures (see Figure 37 on the following pages) [205]. Yet, when there is a lack of efficacy of lantibiotics, it is the result of a low-level resistance that mostly induce innate defense mechanisms rather than lantibiotic-specific elimination by enzymes. For instance, a resistance mechanism prevents these cationic peptides from taking their place in the anionic environment of the bacterial cell wall by incorporating positive charges such as by alanylation of teichoic acids [206]. Other non-specific mechanisms to repel the antibacterial peptides include changes in membrane phospholipid and fatty acid composition, positive charging of phosphatidylglycerol heads or cell wall thickening. Hence, while teixobactin could be subject to these non-specific

[25] They also act via a pore-formation mechanism, whereby they insert their hydrophobic part into the phospholipidic layer, leading to membrane permeabilisation

resistance mechanisms, there is not much to be learned from similarly acting antibiotics to gather intelligence on future resistance.

Relevant resistance mechanisms available in the targeted species

A third way to predict resistance is to look at relevant resistance mechanisms displayed by the targeted strain. Among the two strains tested for resistance by the researchers, only *S. aureus* is discussed here, since *M. tuberculosis* presents specific features that are not encompassed in this thesis. Specific mechanisms triggered by the direct sensing of antimicrobial peptides or indirect sensing of products resulting from cell damage have been identified in this species, although rarely, and result in the expression of a transporter for the active efflux of the antimicrobial peptides [207]. This lead is a very likely mechanism resistance for teixobactin, backed by the fact that several of these transporters have been described in its main bacterial target, *S. aureus*: the BraRS system, essential for resistance to bacitracin, nisin and nukacin, expels the compounds from the cell (Figure 36); the GraRS system is also involved, in strains with diminished susceptibility to nukacin for instance. Involved at the molecular and genetic level, the VraSR system protects against cell wall-targeting antibiotics in general; they induce the transcription of genes encoding important enzymes for the cell-wall peptidoglycan synthesis, for instance murZ and pbp2 [208].

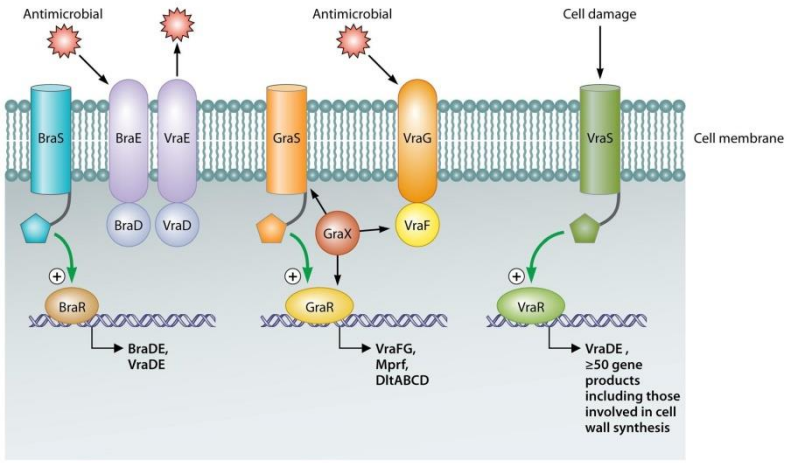
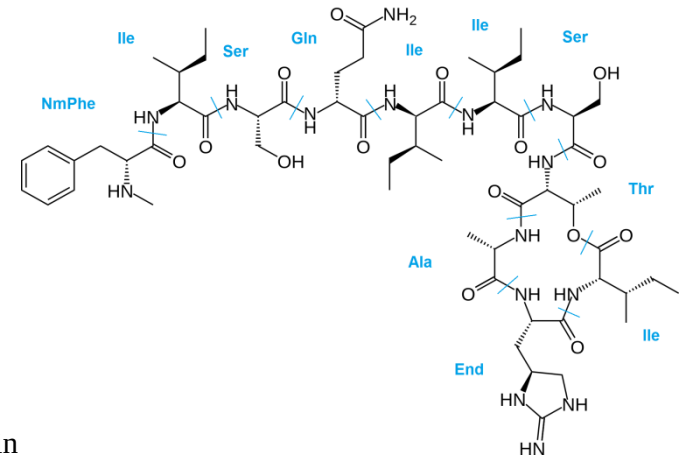


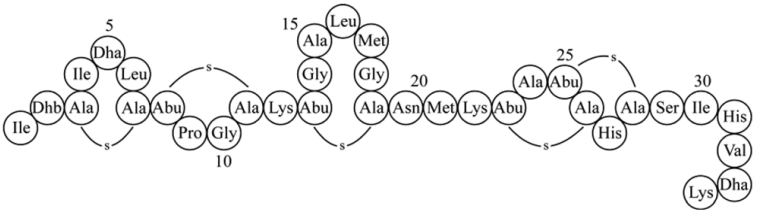
Figure 36. Mechanisms of resistance in S. aureus *specific to lantibiotics. Sensing, signalling, and gene expression are the three steps of these two-component systems (sensor/regulator) that expel antibiotics. From [209].*

On the non-specific side, still in *S. aureus*, it is also relevant to note that the first resistance mechanism that it developed against vancomycin was the thickening of its bacterial cell wall, after having accumulated many mutations in its initially vancomycin-susceptible (VSSA) genome[26]. By

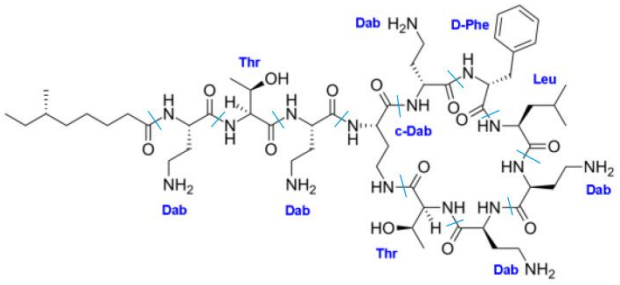
[26] A significant number of mutations are required before reaching the VISA phenotype, and when only a few of these mutations are present, the strain is called hetero-VISA. This hetero-VISA causes refractory infections that eventually yields a VISA phenotype after prolonged vancomycin therapy [350]. Of note, this mechanism is very different from the main resistance phenotype, VRSA, which appeared later on through plasmidic transfer of a specific resistance mechanism (*vanA* gene; see Part 1).



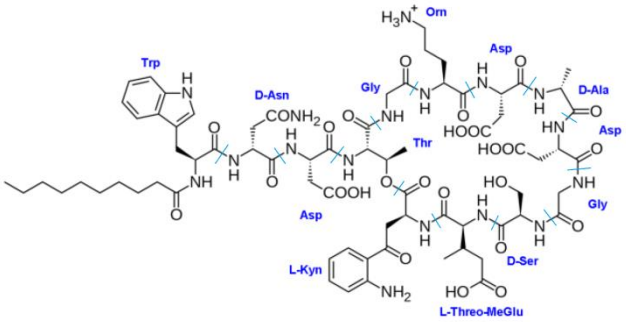
A. Teixobactin



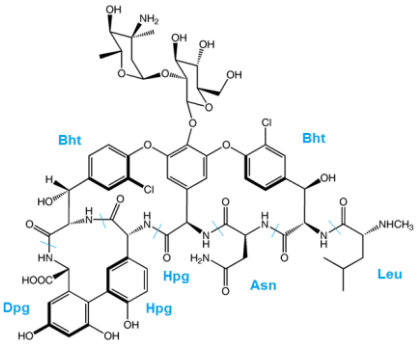
B. Nisin A



C. Polymyxin B



D. Daptomycin



E.Vancomycin

(Previous page) Figure 37. Structures of some antibacterial polypeptides. The abbreviations refer to proteinogenic amino acids except for: NmPhe: N-methyl-phenylalanine; End: enduracididine; Dhb: didehydrobutyrine; Dha: didehydroalanine; Abu: aminobutyric acid; Orn: ornithine; Dab: Diaminobutyric acid; Kyn: kynurenine; Threo-MeGlu: Threo-3-methylglutamic acid; Bht: β-hydroxytyrosine; Hpg:hydroxyphenylglycine; Dpg: dihydroxyphenylglycine; Adapted from [210] and [211].

creating an obstacle to vancomycin penetration and preventing it from reaching its target (in the periplasmic space), the bacteria have developed a decreased sensitivity to the antibiotic, leading to the VISA phenotype [212]. Teixobactin could be subject to this resistance strategy, if the target modification is hardly possible.

In the specific resistance mechanisms category, a few antibiotic-modifying enzymes have been reported in *S. aureus*, such as reductases (nisinase), the aureolysin that cleaves particular human antimicrobial polypeptides, the cathelicidins [213], and endoproteases that remove amino acids from the carboxyl tail of nisin [214]; it is not impossible that teixobactin be targeted by such proteases with a harnessed power.

Resistance mechanisms to chemically similar antibiotics

Fourthly and finally in this argument, taking a broader view at polypeptide antibiotics in general can generate some more insights for resistance development. This family include other compounds such as daptomycin, bacitracin, and polymyxins (polymyxin B, colistin), and while the latter have a broad-spectrum activity against Gram(-) bacteria, they have a structure that relates to that of teixobactin (Figure 37), predicting a potential parallel between some of their most relevant mechanisms of resistance and teixobactin's. Indeed, proteases in the extremely resistant *Burkholderia* genus are supposed to play a role in degrading antimicrobial peptides: the external zinc metalloproteases ZmpA and ZmpB, identified in *B. cenocepacia*, and the periplasmic MucD protease (role unknown) [215]. Although the resistance phenotype *in vivo* is not clearly defined, these genes could be sharpened to effectively inactivate antimicrobial peptides and passed on to other species by plasmids. Resistance to daptomycin, on the other hand, include previously described, non-specific membrane composition and charge alterations (remodelling of the anionic phospholipids in the cytoplasmic membrane), activation of the cell envelope stress response pathway [216] or even biofilm formation [217]; yet distinct hydrolytic mechanisms of inactivation have also been found, including a ring-opening esterase (and a lipase-like removal of the fatty acid tail, since daptomycin belongs to the lipopeptide family, which is not the case for teixobactin). The ring esterase of daptomycin is however very interesting in that it can have practical implications for teixobactin. The enzyme has been isolated from *Streptomyces* spp and is able to hydrolyse daptomycin between the Thr and Kyn residues; it is however not capable of further degradation. Spectrometry analyses have revealed that daptomycin is inactivated by cleavage of the ester bond linkage (Figure 38).

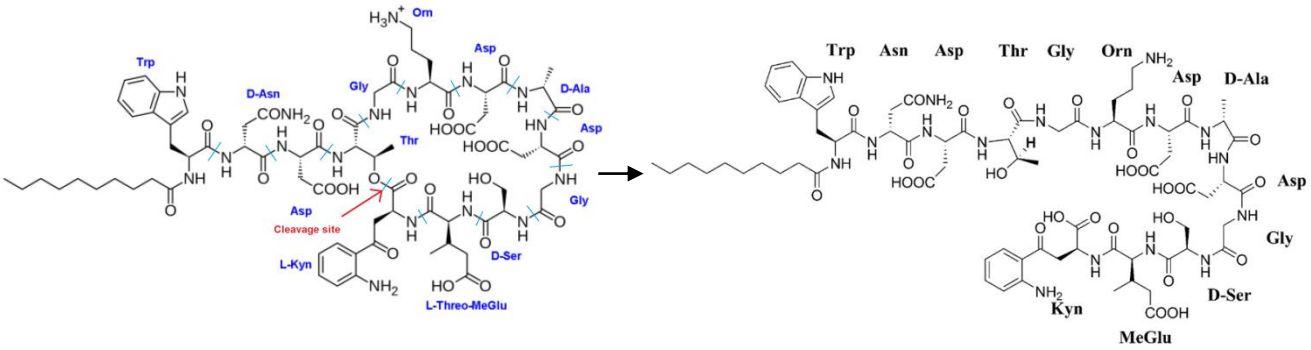


Figure 38. Hydrolysis of daptomycin by cleavage of the ester bond linkage between Thr and Kyn. From [218].

This example reveals a weak point that is exploited *in vivo* by bacterial enzymes and gives an indication of the types of locations where hydrolysis is most likely to happen. Indeed, the ester linkage is thermodynamically more sensitive than the amide linkages between amino acids. Is there a similar weak spot in teixobactin that could be subject to this mechanism? Although the cyclic part of the polypeptide is less important in teixobactin (four cyclised amino acids, compared to nine in daptomycin), an analogy can be drawn between the ester bond in daptomycin, linking Thr with L-Kyn, and the one in teixobactin, linking Thr with Ile (Figure 39). While daptomycin has a nearby reactive, polar aminophenyl moiety to allow and stabilise the cleavage, teixobactin has a less electron-rich structure in the place of the hydrocarbon side chain of Ile. This might be a potentially weak spot for the novel antibiotic, and, should this structure be crucial to the interaction with lipid II, this mechanism would constitute an efficient resistance mechanism.

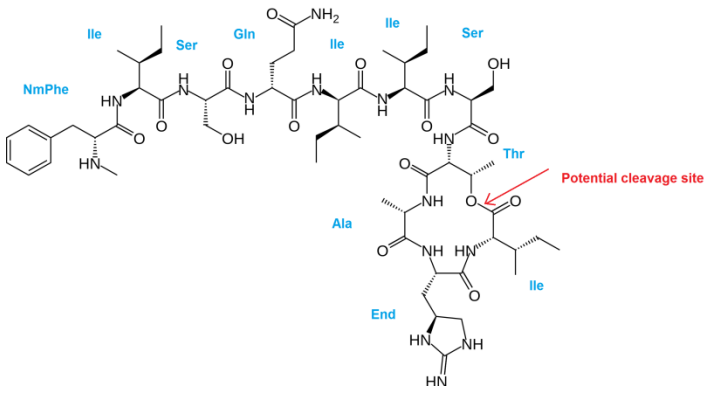


Figure 39. Hypothesis around a hydrolytic degradation of teixobactin.

This mechanism might be chemically probable, yet would it be probable *in vivo*? In other words, how frequent or widespread are proteases for the family of antibacterial peptides? To take a step back, antibiotic-modifying enzymes in the resistance world are mostly encountered for the β-lactams and aminoglycosides families, for which they are among the leading resistance causes. On one hand, β-lactamases are responsible for cleavages of the drugs. They take advantage of the

conserved chemical core of the family, which is also their Achille's heel: the β-lactam ring, which is a weak point that can be opened under the attack of a nucleophilic Serine (for most of the β-lactamases[27]) to the carbonyl group. Such a potentially reactive structure is not easily found in teixobactin, whose main ring is a heterogeneous cycle with an oxygen, three nitrogen, and nine carbon atoms. On the other hand, aminosides-modifying enzymes are responsible for covalent additions on specific OH/NH_2 groups, crucial to the hydrogen-bonding network with their target. Both examples represent the main illustrations for enzymes that modify antibiotics; they do not mean that enzymes against antibacterial peptides do not exist. What are the real-life examples, if any, of enzymes modifying antibiotics more structurally similar to teixobactin (polypeptides)? A first family of enzymes modifies antibiotics by hydrolysis, cleaving the molecule into an inactive one: this is the example of the hydrolysis of daptomycin. Yet this is a new resistance mechanism that has been recently elucidated, although its frequency in resistant strains is unknown and likely to be low (resistance to daptomycin mainly appears by non-specific mechanisms). A second family of enzymes, acting by group transfer (transferases), mainly affect aminoglycosides, chloramphenicol, streptogramins, macrolides or rifampicin. In this list, only pristinamycin IIA (streptogramin A) is a peptide (depsipeptide). So, to answer the questions formulated above, the majority of antibiotic-modifying enzymes are not encountered for peptidic antibiotics, and if some do exist, they are not frequent.

Summing up from the four potential leads

To gather up the evidence available from this benchmarking exercise on specific resistance, peptidic compounds are not very much represented as substrates for such enzymes; for instance, modifying enzymes for vancomycin, a glycopeptide, have never been reported as a resistance mechanism. Hydrolysis of daptomycin by a ring-opening esterase represents the most applicable enzymatic mechanism, due to the chemical similarity, and the most probable one, since the peer group comparison leads to the conclusion that hydrolysis rather than group transfer is more represented for peptides *in vivo*.

Proposal to better identify resistance mechanisms

As a conclusion, all these examples are elements drawn from a peer group analysis aimed at enriching the discussion on the likelihood of resistance to teixobactin through both specific and non-specific mechanisms. For the scientists that introduced teixobactin [200], the method to detect resistance proceeded by single-step low-doses passages or serial passages at subinhibitory concentrations on both species of interest, *S. aureus* and *M. tuberculosis.* The absence of reported resistance points towards either the unlikeliness of such mechanisms or the need for more time to

[27] Another class of β-lactamases employs the mechanism of metalloproteases to cleave the ring.

develop. The latter seems more probable, as both non-specific resistance actions against antimicrobial peptides (cell wall polarisation [219] and membrane composition changes [220]) have indeed been already reported in *S. aureus* – and quite some time ago –, and moreover is the strain known for its antimicrobial peptide-degrading enzyme aureolysin (see above, and [221]).

Using their very new iChip technology to identify resistance would perhaps have been more effective and insightful: indeed, it is very likely that the other soil bacteria surrounding the producer of teixobactin may have already developed defense mechanism against the biomolecule, due to the fact that they live in the same environment. Since these strains have now been made accessible to cultivation, plates with co-culture of competing species could have led to more fruitful conclusions. Maybe even broader resistance possibilities will be revealed once the drug is introduced in the clinic, and caution still has to be put first, before any over-confidence in the efficacy and resistance profile. The importance of conducting a good due diligence on clinical resistance selection should not be overlooked: the family of oxaboroles tells the story of a halted development due to a clinical resistance profile that could have been foretold by properly performed and interpreted studies (this lack of insight amounted to about $170 million being wasted by GSK) [222].

D. PUTTING THE DISCOVERY OF TEIXOBACTIN INTO PERSPECTIVE

Is there a need for more antibiotics targeting the cell wall synthesis?

Targeting the bacterial cell wall has been such a successful strategy[28] that the field is nowadays rather crowded, despite major developments of resistance. Not only does it display the biggest number of antibiotic classes (the well-known β-lactams, together with glyco- and lipo-peptides, fosfomycin etc), it also has a well-stocked pipeline of agents in development [223]. This begs the question of whether there is still a future for inhibitors of the bacterial cell wall synthesis. Among the advances in drugs targeting the bacterial cell wall, the main focus in the past decades has been on further developing carbapenems and cephalosporins via hemisynthesic enhancement, up to the 'niche' point for some compounds active against specific and multi-resistant microorganisms (examples include doripenem, faropenem, ceftobiprole, ceftaroline, oritavancin). The future for this category may see a lot of combination therapies: combinations for narrow-spectrum β-lactam antibiotics (e.g. against *Acinetobacter*), triple combinations (β-lactam, serine-β-lactamase inhibitor, metallo-β-lactamase inhibitor), while "me-too" β-lactam agents may still be viable [224].

Yet the development of more streamlined cultivation techniques and access to previously uncultured bacteria is opening the door to a completely different universe with truly innovative

[28] This is owing to several reasons, including, for the main ones, the nature of the target, easily targeted and with no mammalian equivalent; the great efficiency of the drugs; their favourable safety profile.

molecules and potentially new antibiotic families. Teixobactin is epitomising such a shift and is a vivid proof-of-principle of the value of natural product discovery, when exploiting the great potential of new cultivation techniques (such as iChips). Whether it is going to live up to the high expectations that are set for it will be answered by the additional preclinical tests including extensive toxicology testing as well as the early-phase clinical trials later on. These, according to the company owning the rights, NovoBiotic Pharmaceuticals, are likely to start in 2017 [225][29].

Is there a need for more antibiotics against *Staphylococcus aureus*?

Among the bacterial targets of teixobactin, MRSA is likely to be a major one and may be the main target that the sponsor will seek approval for. MRSA is responsible for a wide range of infections, some even more virulent than its susceptible counterpart (MSSA), yet the question of whether MRSA is still a concern is worth asking in the light of epidemiological data. According to Figure 40, many countries have witnessed a declined incidence, in Europe (from 22 to 18%) and the US (from 53 to 44%) over the past eight years, although the decline has been slowing in Europe [226]. Teixobactin may undoubtedly be an innovative antibiotic, yet the urgency of its need is questionable.

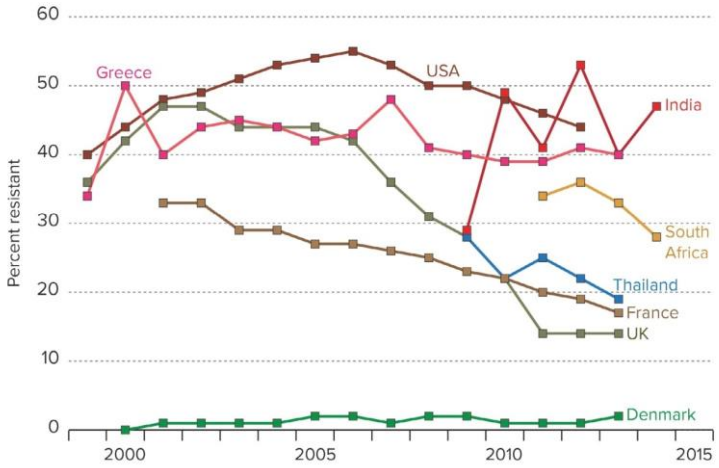


Figure 40. Incidence of MRSA in selected countries (1999-2014). From [226].

As a conclusion to Part 2, antibiotic drug discovery is facing complex scientific challenges, not less associated with the identification routes than with the downstream demanding processes, and rejuvenating the discovery "à la Waksman" will require new cultivation methods and automated techniques. Epitomising the evolution of such scientific challenges is teixobactin, a promising

[29] As of April 2016, no clinical trial for teixobactin was filed with the US registry of clinical trials [351] or the EU register [352].

compound that breaks the records for innovation: this new molecular entity was not only discovered in a previously unknown bacterium, thanks to a novel cultivation method, it also marks the birth of a new antibiotic family, the (cyclo)depsipeptides. All things considered, teixobactin is the silver lining that proves that still in 2016, adapted and innovative natural routes for drug discovery are (one of the) potential answers to the scientific challenges of antibiotics. Whether the other aspects of the antibiotic industry, namely economic and regulatory, can also be conducive to innovation is the second essential element that will be discussed in the next part of this thesis.

PART 3: ANTIBIOTICS: WHY MARKET FAILS.

THE ECONOMIC AND REGULATORY PERSPECTIVES

As emphasised in Part 1, antibiotic resistance represents a public health threat with economic consequences looming at the horizon. Moreover, pharmaceutical companies have stated their dissatisfaction with the antibiotic market [227] – and society has witnessed the consequences: there are very few novel agents on the market or in the pipeline. To address these problems and generate more business-friendly models to enhance antibiotics development, it is useful to understand why and how has the situation reached this critical point. The main hypothesis developed throughout this thesis is that the market failure for antibiotics is the consequence of a flawed business model combining tough scientific grounds, unattractive economics, and regulatory hurdles.

Whether the attrition rate in antibiotic development is more strongly correlated with the scientific challenges or with the environing conditions (regulatory, economic) is complex to answer; yet, regardless of the factor that is most to blame, there may be a need not only for entirely new discovery models as new sources of antibacterial drugs for the industry [200], [228], [229], [230], but also for regulatory evolutions on the health agencies' part to back their development and commercialisation. Therefore, to complete the picture on the antibiotic industry and its business model are the economic specificities of antibiotics, the regulatory component, and economic incentives discussed in this third part, highlighting the interconnection of the different pieces of the antibiotic business model puzzle.

Applied to teixobactin, this Part endeavours to address the question of whether it would be more effective to integrate the drug in the current business environment, with its characteristics and industrial actors (Section 2), or to develop it within new frameworks that answer to the current inefficiencies (Section 3).

SECTION 1. ANTIBIOTICS: A (VERY) PARTICULAR CLASS

A. A DEFINITIVE ACTION

A first characteristic sets antibiotics apart from many other therapeutic classes. The fact that these drugs actually cure the disease they are prescribed for (since they kill the responsible agent: the bacterial pathogen), makes them stand out, as most medicines do not do so: on the whole, drugs

only act by relieving the symptoms (with the exception of some antineoplasic or antiviral treatments).

Additionally, antibiotics treat infectious diseases that are, by definition, acute conditions, so the treatment is to be short, which has economic consequences in the form of limitations of prescriptions and volume generated. In addition to their definitive action, the fact that they also usually act in a few days, most of the time in an impressive manner, and with few side effects, earned them the designation of 'miracle drugs' in their early years. Other antimicrobial agents would have had that kind of reconnaissance too, for example vaccines, where it not for the fact that they are prophylactic agents, and their impact, far from being impressive and immediate, is rather thankless and of no explicit reward; because therapeutic gains from vaccines are measured on one or several generations, not to mention the mediatised adverse events and possible associations with serious complications, their benefits are clouded and obscure to the layperson. Nevertheless, the mechanism of action of antibiotics is of particular consequence to their economic and business model, as demonstrated in the rest of this section.

B. A DIFFERENT TARGET

A human receptor or target (taking the form of any biomolecule such as a protein or a nucleic acid) and the intention of acting on a specific physiological pathway within a patient are the alpha and the omega of classic therapeutic science. This is the rationale for drugs as the public knows them, in their most general form, from psychoactive to antihypertensive drugs, including antineoplastic, bronchodilatory or anti-inflammatory drugs.

Yet, when it comes to antibiotics in the most general sense ("agents against (external) life", against microorganisms), and antibacterial drugs in particular, the target is a very particular one. Not only are bacteria living organisms: they have a life of their own, contrary to viruses which need to infect a cell and hijack the host's cellular machinery for their growth, and they can thrive independently, contrary to parasites that need to seize or exchange external materials with species in their environment. This resilience and versatility may explain why they are among the oldest living organisms on Earth (see Part 1, Section 2 A), and therefore the most well-equipped species to survive. Targeting such adaptable and cunning organisms is hence no pleasure cruise, also rendered harder by the escape mechanisms that these species have developed through first-hand experience.

C. A UNIQUE, SPECIFIC CONSEQUENCE OF THEIR USAGE

Unlike virtually all other therapeutic classes (and making them once again special drugs), antibiotics are intrinsically and naturally vulnerable to resistance, which is not a disease, not a side effect, not

a particular condition of the patient, but really a specific, evolutive status of the pathogenic agent, inherently linked to the fact that these are living creatures are capable of high adaptability. Bacteria are microorganisms that have been around for billions of years and are the most skilled at developing solutions and adapting to hostile environments. This constitutes the roots of the resistance problem. Indeed, when a population of bacteria is faced with the cytotoxic power of an antibiotic, only the ones that have such a selective advantage as to allow them to survive the attack will live on. Resistance impacts every level of the infectious disease and antibacterial therapeutics sphere: from the very molecular mechanisms that threatens the killing potential of the drug, and therefore its efficacy in curing the infection at a phenotypic level, to the macro view encompassing the general evolution of bacteria as a species and the public threat to the globalised population, while being also a burden for the world of the pharmaceutical industry. The latter must indeed contend with an intrinsic "self-destruct" mechanism that, regardless of the timing of its apparition, whether after two years or two decades of clinical use, will eventually emerge, putting an upper boundary on the drug's therapeutic lifetime, as resistance is not a matter of *if*, but *when*. For a development process that is complex, time-consuming (10-12 years to market) and expensive (requiring an investment of more than $1.5 billion) [231], the returns, which are already risky, are further impeded by a commercial life shortenend by resistance.

To take a more optimistic view, resistance can also have, if not a bright side, at least an industry-friendly consequence. Although resistance is a phenomenon that might limit the clinical usefulness of the drug, what nevertheless comes out of the drug's finite efficacy in front of the pathogens' ever-renewed array of resistance mechanisms is the absolute guarantee of a market for further antibacterial drugs. A target that is continually evolving results in a never-ending demand for sharper and more effective weapons.

Both features have therefore notable consequences on the economics of antibiotics, as pictured in Figure 41: whether this paints a gloomy or just a different picture, it is of little doubt that the business model for antibiotics is quite unique.

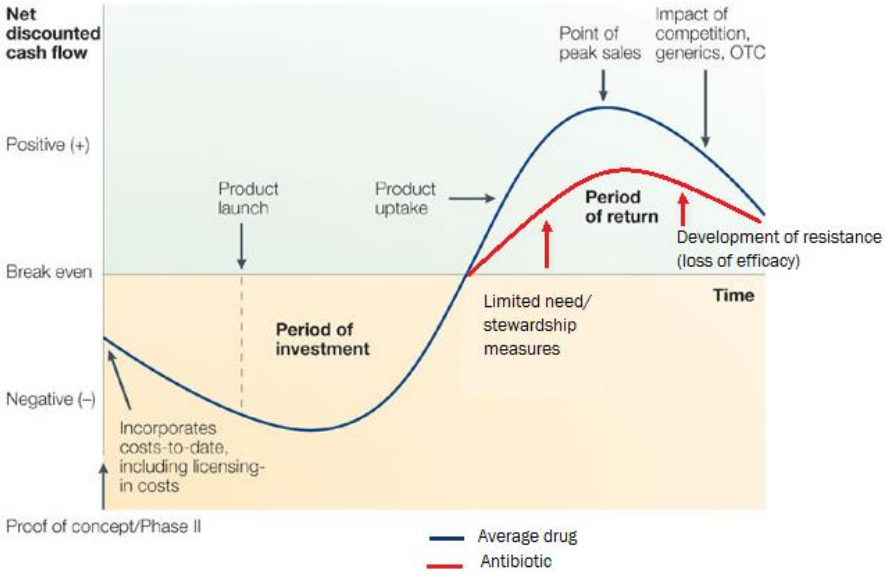


Figure 41. The antibiotic business model in the 21st century. Adapted from [232].

D. A PUBLIC GOOD

Antibiotics are also particular in that their usage does not only impact the treated patient. The population at a broader level also experiences the consequences of any antibiotic usage, whether it be appropriate or ill-intentioned. This characteristic of a link between individual and collective consequences has earned them their stamp of "public good" products (or "societal drugs").

At the core of their public good property lies an economic concept called externalities[30]. An externality arises when actors (in economic terms; firms or individuals) do not account for all the benefits (positive externalities) or costs (negative externalities) of their actions. Because antibiotics address communicable diseases, similarly to vaccines, one patient's use affects the drugs' efficacy for everyone else; therefore, the outcomes and consequences (the costs and the benefits) are not solely borne by the patient [233].

Moreover, of particular interest with antibiotics is the fact that these externalities can be of both types: either positive or negative. Indeed, when a patient takes their medicine with enough compliance to eradicate the bacterial infection, they stop being a contamination risk for healthy people, as they are not infected anymore and therefore help decrease the risk for others to become ill. The same type of *positive externality* happens with vaccines, for instance, where the immunisation of patients decreases the risk for the rest of the population to contract the viral disease. However, and unlike vaccines, antibiotics also display *negative externalities*: other people will

[30] Analyses of antibiotics (and the issues associated with them) in economic terms (as economic agents) are rarely undertaken among the leading scholars in the field. This is however an important basis as by deductive reasoning there are a number of sensible solutions that economics can contribute to the field. See for reference [353], [371], [288].

suffer the costs and adverse outcomes of somebody's misuse of antibiotics. There are two possible situations bringing about negative externalities. First, if a patient does not take their medicine as prescribed, by missing doses or shortening the treatment duration, not only will they not eradicate the infection and consequently be an infectious hazard for healthy people, but they will also impose a selective pressure on the bacteria, educating them into resisting the treatment. This means that when other people will contract the infection with these particular bacteria, the standard drugs of care will not work, and the outcome of the disease will depend on the availability of second-, even third-line treatments, which for some strains is far from guaranteed. Secondly, on top of this first detrimental consequence is another negative externality very specific to antibiotics and to the nature of their target. These newly resistant bacteria can then infect other patients, but the problem does not stop here. One of bacteria's remarkable answers to survival is their ability to *transfer* their resistance genes, to pass on to other strains the selective advantage that enabled them to survive, were they to need it one day. The selective advantage can take several forms, encoded by a resistance gene that can be released to other bacteria through transformation, transduction or conjugation (see Part 1). The consequences are environmental: the negative externality of a misuse of antibiotics will translate not only in resistance to the initial strain (first consequence), but also of similar, neighbouring and environmental strains (second consequence) (Figure 42).

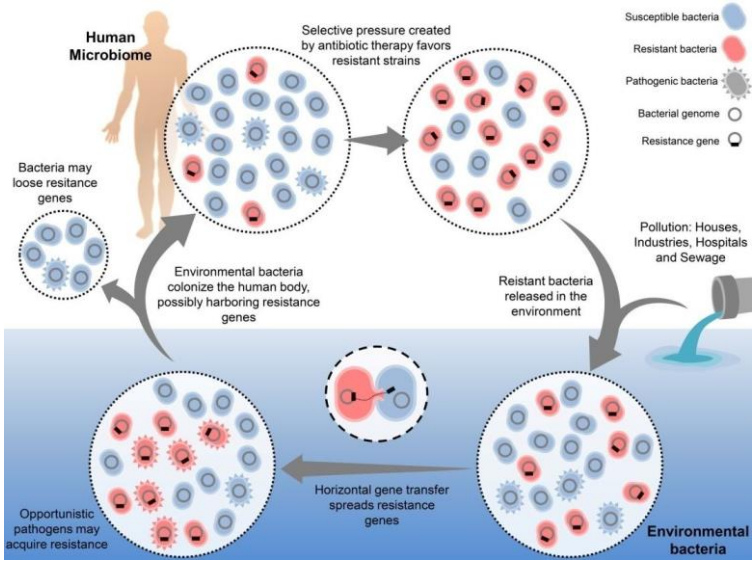


Figure 42. A consequence of the negative externalities of antibiotic usage: the environmental dissemination.

Antibiotic misuse and its main consequence – resistance – is a societal problem: This 'tragedy of the commons' [234] is a classic example of how individuals — acting rationally and according to self-interest — can damage or destroy communal property. The idea is often used to frame environmental issues, but it also has resonance for bacterial resistance. In case of overuse or

problems relating to antibiotics, the perceived cost to the individual is small, whereas the actual cost for society, cumulated, is quite large.

"One person's use – or abuse – of an antibiotic could be another person's demise", to borrow the words of leading figure for antibiotic awareness Dr. Stuart Levy [235]. Two opposing externality forces are at stake with antibiotics; the economic concepts imply that not only the social benefit (positive externality) but also the social costs (negative externalities) are greater than the sum of individual benefits and costs. When it comes to the positive externalities, the overall market demand for antibiotics (private demand), because of its shortsightedness and absence of valuation of the broader benefits, will be too low compared to the social optimum (the general public, as lay persons, do not realise how useful antibiotics are, beyond their individual efficacy), and a low demand results in a short supply and a low price. Because the price is lower (than in the absence of externality), there is a lower incentive for the industry to enter this market and produce these drugs. On the other hand, there are caveats to mitigate this reasoning: demand, in the particular setting of healthcare, is not merely defined by the preferences of the individual (the patient); rather, physicians' preferences define demand. As it is believed that, with their education and professional awareness, they understand and have incorporated the greater good of antibiotics, they would be able to correct the market deficiencies and increase demand to its optimal level. Only this requires that physicians have the correct understanding of infection transmission, and that they act upon it, but these conditions and the extent to which they weight in a physician's prescribing decision cannot be ascertained.

To sum up, the misuse of antibiotics confers risks upon the individual and the wider community. These risks include the selection of resistant and more virulent infections, opening the door to opportunistic fungal infections, and leading to subsequent, more drug-resistant rounds of bacterial infections. Similar trends can be seen at the organisational level. Is there a failure of collective action when no individual insurer will invest in an infection control policy at a local hospital? Would a drug company show self-restraint in marketing an antibiotic, when its therapeutic competitors do not, just to factor in the economic rationale behind a right antibiotic usage? It is a very consequence of their 'public good' characteristic that antibiotics require collective action, or state intervention, to deter their low-value uses and preserve effectiveness for their future high-value uses; and when the political sphere starts to take charge, as evidenced by the current political agenda outlined in Part 1, can one hope for the market failure to start being corrected. The second solution to respond to externalities is to internalise the external costs into the price, and this will be discussed with the original economic proposal at the end of this part.

SECTION 2. THE STRUCTURE OF THE ANTIBIOTIC MARKET AND THE COMPONENTS OF ITS MARKET FAILURE

Market structure has a big impact on antibiotic use. A failure of the market, rather than signifying the complete inadequacy of the market structure, highlights some major inefficiencies. Indeed, some companies are still conducting R&D efforts or entering the field (see Appendix 7, Tables 7-8), while some specific problems on the other side (developed below) are not addressed by the current trade structure.

After a characterisation of the current structure, a snapshot which highlights the industrial players and the antibiotic market failure, the main components responsible for the current situation, namely the scientific, regulatory and economic aspects of the development of antibiotics, are further analysed, elements whose crucial interconnectedness is essential to recognise in order to improve the prospects for the industry and to ensure a prolonged efficacy with a public health gain.

A. WHAT IS THE MARKET FAILURE IN THE ANTIBIOTIC INDUSTRY?

a. The structure of the market and the organisation of the industrial actors

If one were to successfully develop and commercialise teixobactin, one would wish to know how the market for antibiotics is articulated, in order to maximise its return on investment on the drug. What is the most sensible player to engage with, and at which stage? What is the business model of a company developing antibiotics in 2016? What is the industrial organisation and market solution to most efficiently bring a novel antibiotic to market? What is the current optimal business structure to market a new antibiotic, and, to put this into practice, what conclusions can be drawn for a successful development of teixobactin?

To summarise the business plan in a sentence, drug companies are engaging in a field where a particular characteristic resulting from the mere use of their compound – resistance – renders their drug obsolete, in an inevitable yet unpredictible way, creating a fundamental uncertainty on the size of the market and the duration of their drug's effectiveness. Yet some companies are still engaged in this field, and some small biotechs are even founded to focus solely on antibiotic drug discovery.

Where do we come from?

It was an extraordinary change that the antibiotic market has witnessed. In 50+ years time, the drugs went from trials in real life and physicians' testimonies of efficacy to stringent and formally guided clinical trials; from over-the-counter distribution and heavy, sugar-coated adverstising to plain scientific objectivity and a stewardship gearing towards more and more rigorism, with prescribing

rules to limit irrational use; and, as a parallel (and a consequence), from a cherished prescribing liberty that the 1950s physicians held very dear, a "right to irrationality" that would define their corps, to guidelines and controlled therapeutic pathways, insurances and health maintenance organisations that little by little take the provision of care away from physicians – and would have led to an immediate outcry in the antibiotic's golden era [236].

While these environmental changes are not exclusive to the antibiotic industry, they have led to incremental evolutions essential in impacting and shaping today's business model of antimicrobial drugs. The status of physicians as well as the public view of the pharmaceutical industry as a whole have changed, from an industry saving the lifes of the nation's soldiers with their 'miracle pills' to a lucrative business with an ever-escalating race to outprice a competitor's oncology drug or 'mAb' therapy (monoclonal antibody).

Current players in the antibiotic field

Current sales for antibacterials represented $13.4 billion in value, and are forecasted to grow only by 1.4% per annum until 2020 [237]. Despite this value, antibiotics are not currently seen as attractive to big pharmaceutical companies, nor are they valued very much: while the rankings of the most valuable R&D projects feature chronic diseases such as hepatitis C, cystic fibrosis or lung cancer, no antibiotic medicines are stated in the Top 20; similarly, and on an even broader consideration, antibacterial drugs are lagging behind the most profitable therapy areas in a sheepish 15^{th} place [237].

The number of companies active in the antibiotic field, as well as the number of antibacterial drug discovery programmes, have plunged, as illustrated in Figure 43. This is due shifts in R&D programmes to more profitable therapeutic areas, and may also have resulted from the consolidation of the industry since the late 1990s; mergers and acquisitions among pharmaceutical companies have led to a loss of research groups with focus and expertise in antibiotic drug discovery. Illustrating this is the fact that Merck &Co., subsequent to its acquisition of Cubist in 2015, pulled the plug on the company's early R&D operation, which also resulted in the dismissal of 120 Cubist researchers [238].

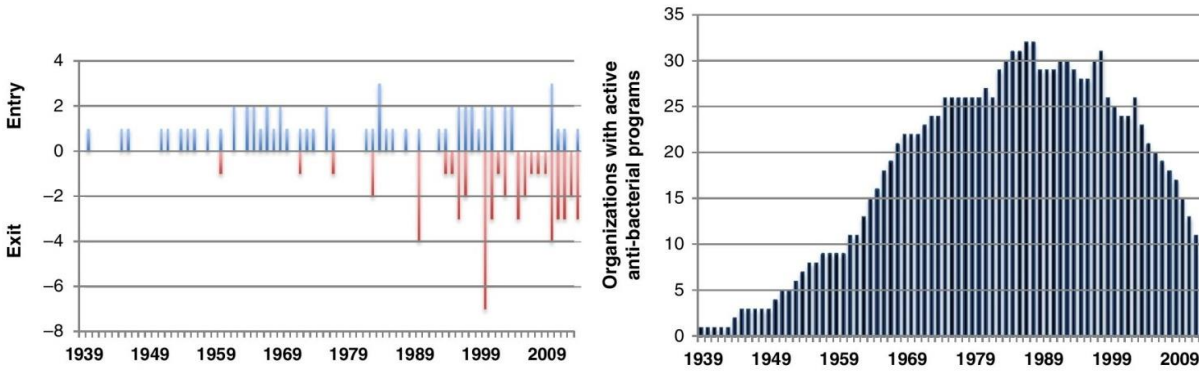


Figure 43. Evolution over time of the number of corporate entries and exits in the antibiotic therapeutic area, 1940-2013, and the number of companies that obtained at least one new antibacterial molecule and remain active in the field. From [239].

Biotech and big pharma's interplay

If major pharmaceutical companies are exiting the field, what about smaller biotechnology companies? Indeed, several smaller companies have stepped in to fill the void and are focusing on the development of antibiotic compounds (e.g., Cubist Pharmaceuticals[31], Basilea, Paratek, NovoBiotic or Oscient) (see Appendix 7). How can it be explained that these companies manage to find some attractivity to the antibiotic field?

First, these small structures have an expertise resulting from their own history to develop antibiotics. Results from a European survey concluded that most of these small and medium enterprises (SMEs) have a background in an academic setting, with the spin-out of a company from an academic laboratory subsequent to the discovery of a promising lead compound, or the incorporation by experienced industry experts licensing a new technology [240]. The main reason that motivated the creation of these enterprises related to the prospect of taking further an opportunistic discovery by developing it in a creative and innovative manner and contributing to human health.

Yet, when it comes to the second point, the scientific heads, the picture is more blurred. A particular challenge facing the biotech companies active in the antibacterial field today is the lack of highly knowledgeable, competent and experienced people. As the bigger pharmaceutical companies started to exit the field decades ago, the supply of trained scientists and chemists diminished.

Thirdly comes the question of the necessary financial muscle, as antibiotic biotechs are also facing these non-specific, financial SME-related challenges. Although their structures are more cost-effective and flexible (fewer overhead costs) than bigger firms, with a different business model entailing lower R&D costs and no blockbuster-type portfolio, seed capital is an essential prerequisite. Firstly, because of their dependency on one product (or sometimes class of products),

[31] Cubist was acquired by Merck in January 2015.

biotech companies are not optimally hedging their development and marketing risks, and the failure of one drug development programme can threaten the existence of the company. Secondly, the organisation and symbiotic relationships in the pharma industrial landscape are typical: in order to advance new classes of antibiotics from discovery to development, they may need the financial support of larger companies or other backers to fund late-stage clinical trials and commercialization. As much as they may depend on university and academic institutions for their initial technology, biotech firms depend on large pharmaceutical companies for the advancement and ultimate marketing of their product. This traditional view is no exception in the antibiotic market. Therefore, the next challenge is to guarantee such financial back-up from big pharmas, which can take several forms – from the licensing-out or sale of a particular asset to the complete sale of the business. Yet the major pharmaceutical industries, like all other publicly traded industries, must deliver for their shareholders in order to justify their continued investment and are risk-averse; hence, the unique nature of antibiotics makes exit opportunities and, consequently, securing investments challenging. Because they produce a weak return on investment for manufacturers (antibiotics work so well and so fast; see Section 1), pharma is disinvesting the antibiotic field as it currently stands. Indeed, large pharmaceutical companies require annual sales of $500−800 million in order to recoup R&D costs, whereas many SMEs need substantially lower annual sales to recoup investments, perhaps $100−200 million per year [241].

To sum up, the organisation that generally rules the pharmaceutical field as a whole applies for antibiotics as well. SMEs focus efforts on developing and bringing to market previously discovered molecules rather than discovering new targets in-house. SMEs endure high costs when taking a new drug to market and thus have limited financial resources left to invest in new drug discovery efforts or basic research programmes, which posits the issue of the sustainability of such a business model for antibiotic drug discovery. As a general rule, which again applies to the antibiotics market, most SMEs are dependent on venture capital firms (VCs), ‘angel’ investors and public funds for their initial, start-up phase, but access to private funding can be problematic for several specific (antibiotic-related) and non-specific reasons (Table 6). These concerns have translated into a substantial 28% diminution of global venture capital in antimicrobial R&D between 2004-2008 and 2009-2013[32]. The most common incentives were direct project funding, research collaborations, and research grants and fellowships for scientific personnel[33].

[32] Based on 2008-13 data from the Biotechnology Industry Organization (BIO) report, $181 million was raised annually through venture capital for global antibiotic R&D [370].
[33] Renwick et al have estimated that European public funding amounted to c.€147 million per year between 2007 and 2013 (for the R&D of antibiotics, alternative therapies and diagnostics), whereas their US counterparts invested c.$260 million (c.€240 million) in antibiotic R&D in 2015 [313].

Barriers to access to capital
• Little expertise of the VC firms in the antibiotic field, hence not fully realising the potential value • Herd behaviour from VC firms following well-known, trending therapeutic areas for investment • Investors deterred by lower prices for antibiotics compared to other pharmaceuticals • For private capital available in anti-infective field, potential sales considered lower than for other, chronic anti-infective treatments (e.g., hepatitis C) • Absence of a well thought-through business model • Risks and returns not clearly evaluated or, if ROI was calculated, number was meaningless • US private capital: not readily available to EU firms, as deterred by the pricing & reimbursement systems & HTA agencies

Table 6. Financial barriers to investment in antibacterial small comapnies. VC: Venture Capital. HTA: Health Technology Assessment. From [240].

Outliers

Charity is another actor in the pharmaceutical landscape. Less weighty than traditional industrial powerhouses, they have the specific purpose of addressing the remaining market failures in drug development by furthering the development of drugs that are needed from a social, public health point of view but also have such a poor profitability profile that they do not fit into the traditional business model of the pharmaceutical industry. The Bill & Melinda Gates foundation is an example of action in various areas of global health (HIV, malaria or neglected tropical diseases) where the foundation, among other actions, supports the development and delivery of new drugs, vaccines or diagnostic tools. This approach does not mean that the bigger actors are completely excluded from the actions taken; on the contrary, many of the top 10 drug companies finance charitable efforts at arms-length or support independent charities that are in charge. Examples include Bristol-Myers Squibb and Pfizer's efforts against global AIDS, AstraZeneca's tuberculosis drug discovery initiatives of GlaxoSmithKline's support of the DRIVE-AB initiative for the discovery and development of new antibacterial drugs.

Can an antibiotic still be a blockbuster in 2016?

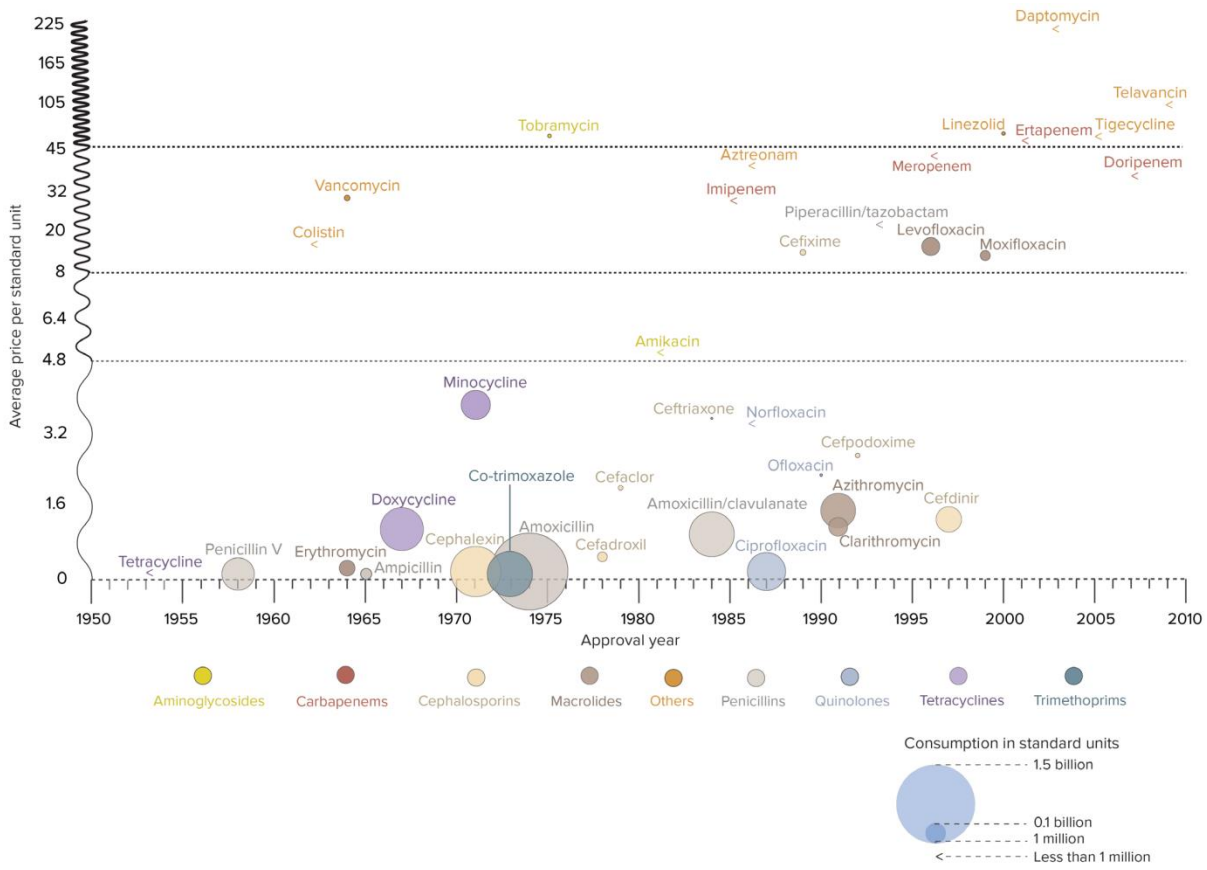


Figure 44. Prices and consumption of selected antibiotics in the US by year of FDA approval (2010 data). From [226].

If there is anything that Figure 44 tells about the commercial returns of an antibiotic, it is that they are very disparate.

On one hand, some antibiotics have reached the status of blockbuster. For instance, Zithromax, the best-selling antibiotic in the world in dollar terms, had U.S. sales increasing steadily, from $104 million in 1994 to $1.8 billion in 2004; over the same period, Zithromax has increased its market share among all antibiotics and within its class (macrolides) from 1.3% to 11.4% and from 15.1% to 68.7%, respectively (Outterson's analysis of IMS data, in [242]).

On the other hand, some recently approved antibiotics and some current agents in development look much more like corporate social responsibility than good business. Johnson & Johnson's recent drug for multiple-resistant tuberculosis, bedaquiline, has a small market (it was indeed designated an orphan medicine); only people who have failed other lines of treatment. It should not be overlooked that orphan drugs can be profitable despite their small market, yet when adding the characteristics of the antibiotic market on top of it, such as keen stewardship and restricted use, the commercial proposition looks much more difficult to defend.

When antibiotics are indeed priced highly, there is also the issue of HTA and reimbursement bodies. For instance, in 2009 Canadian Expert Drug Advisory Committee (CEDAC) recommended that daptomycin (Cubicin) not be listed as reimbursable for the treatment of complicated skin and skin structure infections and Staphylococcus aureus bloodstream infections, citing, among other things, its high cost of $165 per day (compared to existing alternatives: $92 for vancomycin or $141 for linezolid) [243]; consequently, the Executive Office decided not to fund the drug.

Yet, and it was before this setback, Cubist Pharmaceuticals had positioned their Cubicin (daptomycin) as a future blockbuster, anticipating more than $1.8 billion in sales in six years from launch (Figure 45). Its actual total sales for the drug were $967, $860 and $736 million for the

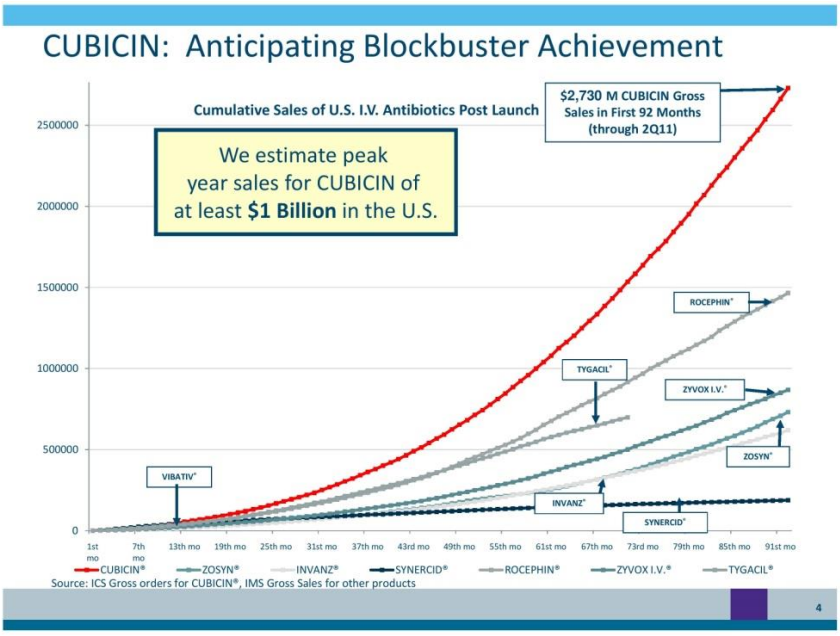


Figure 45. Cubicin (daptomycin)'s projected sales. From Cubist Pharmaceuticals, dated September 2011 [246].

2013, 2012 and 2011 fiscal years respectively [244], while for 2015 Merck & Co. reported a figure of $1,127 million [245].

Hence, historical data shows that antibiotics could indeed be blockbuster. Yet these success stories of broad-spectrum agents may not repeat in the current or near-future environments: with much narrow-spectrum agents, much scrutinity from health agencies and an unprecedented political awareness, it is unlikely that history will repeat. Regardless of whether it will, the question should focus on whether it should. Advocated here is the view that antibiotics should not be blockbusters. They should not be subject to high-volume sales and heavy marketing campaigns, for the reasons that are well known known, that a wider use will result in more and more resistance.

b. What is the market failure in the antibiotic industry?

Market failure is a recurrent theme in health economics, as several characteristics and unique features of this field explain why basic market mechanisms and traditional economic principles cannot apply. For example, in the market for health insurance, assumptions about probabilities of ill health do not satisfy the general assumptions of insurance theory, resulting in the absence or poor development of the health insurance market [247]. Several features on which the health care market differ from the ideal economic market include asymmetric information between physicians and patients along with relationships of trust that go against the principles of impersonal transactions, public goods, and externalities, situations of monopoly with government implication or differentiated and heterogeneous products and services [248].

Pharmaceuticals, in particular, are no exception to this departure from classic economic theory, as informational imbalance with regards to medicines efficacy, quality or safety is dominant between the patient and the prescriber, but to a certain extent between the manufacturer and the prescriber as well, which could lead one actor to take advantage of the situation to enhance their profit, for example. In contrast to the overall health care sector, the pharmaceutical sector displays substantial issues related to the failure of competition as well. Specific characteristics such as research and investment (R&D) costs, scientific capabilities, regulatory expertise are as many barriers to entry that are balanced by intellectual property rights, brand loyalty, market segmentation and collusion tactics that can happen at different phases of the product lifecycle [249]. But within the pharmaceutical category, antibiotics are an even more special case for market failure, for several reasons developed here.

The failure of the market does not mean that no antibiotics are produced at all or that all the players have exited the field (the list of companies still engaged in antibiotic R&D in Appendix 7, Tables 7-8, indeed tells otherwise). Rather, the observable consequence of a market failure for antibiotics is that the market is not producing drugs *efficiently*, either because not producing enough drugs or not producing drugs that meet all of society's needs (for instance, the much-needed antibiotics active against Gram(-) organisms; see Part 2). The industry may produce antibiotics that are not delivering the highest utility to society (low-value antibiotics within the antibiotics segment, such as with the predominance of me-too compounds over novel chemical classes), or may supply fewer antibiotics overall than needed to address the threat of resistance (too few antibiotics within the pharmaceutical segment). What are the reasons behind these market failures? Antibiotics, as pharmaceuticals with special features (see the above Section), require special economic analysis. Conjugating the special characteristics of the subject at the three moods that structure the antibiotic industrial environment (scientific, regulatory and economic) brings some answers.

B. SCIENTIFIC

In an order that does not reflect the relative importance of either factor, the first reason to explain market failure is scientific: antibiotics have specific therapeutic and clinical features that not only make them hard to discover, but also do not always make them attractive to develop. This paragraph only serves as a reminder of the approaches and challenges to antibiotic drug discovery, lead development and resistance that Part 2 has specifically developed. On the bright side, the science behind antibiotics also has some attractive features, whether it is their usually low toxicity when targeting bacterium-specific biomolecules, further reduced by short treatment durations which also lower the downside risk of drug development.

If this scientific component is a technical challenge not to be downplayed, it is, however, less subject to useful and impactful paradigm changes than the two others: indeed, basic scientific research, regardless of the economic environment in which it occurs, still has to be conducted with its core principles, and resistance is inevitable. The policy actions to stimulate antibiotic development do so by addressing the two other components of market failure, on the basis that they either increase public sector funding, tax credits, innovative inducement prizes and other subsidies; or reduce regulatory costs and leverage IP rights. The main elements of and proposals on the regulatory and economic sides will be analysed in the dedicated sections below.

C. REGULATORY: REGULATION ON ANTIBIOTICS AND THE IMPACT ON INNOVATION

Another important feature of the antibiotic market lies with its regulation. The drug industry, in general, is highly regulated, with the institutions' specific requirements, approval mechanisms, and market control being as many barriers to entry, which are briefly reminded here[34]. Quality, safety, and efficacy have always been the basic premises that any drug is required to demonstrate, and regulatory bodies to ascertain with their seal of approval, through *in vitro*, preclinical and clinical trials; the tracking of the apparition and evolution of adverse effects even continues after the marketing phase in real-life settings. Increasingly are governments paying attention to the cost of the medicine delivered to their population via the more and more budget-constrained healthcare systems; this has given rise to requirements of cost-effectiveness, the "fourth hurdle", where another layer of proof is incumbent on the drug company to demonstrate the relative economic advantage

[34] For a comprehensive review on the regulatory requirements ruling the world of pharmaceuticals see [344]. The requirements on quality, efficacy and safety can be found with the International Council for Harmonisation [354], while the FDA and EMA, the main regulatory agencies, also provide detailed information about their area of expertise. On drug regulation see [356], and for the topic enriched with most recent issues and evolutions of the field, see [355] and [358]. On the growing place of health technology assessment and economic evaluation requirements see [359].

of using their drug, usually within a specific pathway or for targeted illnesses. Embodying this concept, providing guidance and voicing support for more economic regulation are dedicated institutions such as the NICE in the UK or the CEESP in France (while the US are remarkably lagging behind). With the review of dossiers progressively maturing are additional points taken into consideration, such as risk management plans (RMPs), post-authorisation commitments and moves towards adaptive licensing. A more granular view on antibiotic regulation itself, with a focus mainly on the EU and US systems, can be taken to identify to how and what extent the regulatory systems can further or inhibit the development of antimicrobial drugs.

Regulation on antibiotic compounds had been identified by some specialists as another explanation for the failure of the antibiotic business model. Backing this assertion is the trend in antibiotic approvals that, despite the drug discovery potential and the need for new antibiotics, has been showing a decline over the past decade, with the exception of the last couple of years: Figure 46 shows the number of antibacterial New Molecular Entities (NME) approved between 2004 and 2016 [**250**].

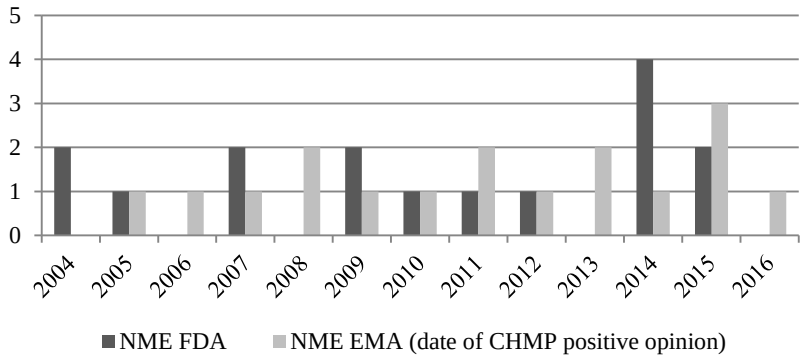


Figure 46. Antibacterial NME approved by the FDA and the EMA (CHMP), 2004-2016 (data as of April 2016).

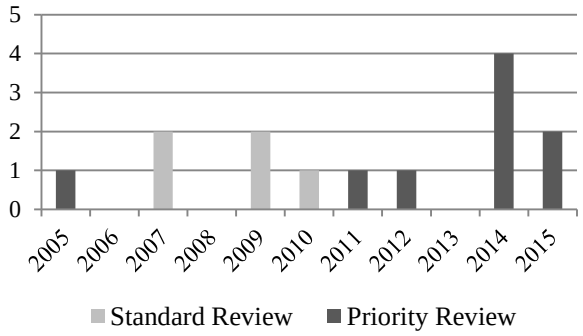


Figure 47. Breakdown of FDA antibacterial approvals by type of review (data as of April 2016). Note: all the Priority Review approvals in 2014 and 2015 are QIDP drugs. Sources: FDA.

Of particular interest are the trends in types of approvals, displayed in Figure 47: antibiotics are increasingly approved through priority review and other earmarked procedures, such as the recent QIDP (Qualified Infectious Disease Product), described later on in this paragraph.

The insider view: considerations when discussing approval for a novel antibiotic in 2016

There are classic concerns and issues that are put forward to explain the regulatory hurdle that antibiotics face (clinical trial requirements, for instance; these are discussed below). Yet in order to better understand the actual, real-life challenges, it is useful to gain more insight into the regulatory approval process and decision as they happen in 2016, to identify the particular points that the drug agencies are most attentive to. The most recent FDA committee discussion on the new drug application of a novel antibiotic[35], the ceftazidime-avibactam combination, Avycaz (Cerexa) is quite informative to this end [251]. A lot of the current issues with antibiotics are indeed recognised as such by the FDA committee: some voting members approved the drug because of the unmet need – "it is another tool in the box and we are desperately in need of them" – while other proposed to add resistance as a third element to the FDA's field of regulatory competence, alongside efficacy and safety. The members were also striving not to rush their judgment on the basis of an unmet need when all the necessary data were not available. Hence, Avycaz received approval for complicated intra-abdominal and urinary tract infections, based on the preliminary results of a Phase 3 trial (intra-abdominal infections) and Phase 2 data (urinary tract infections), although it was recognised that the data were not as robust as usually needed (the unmet need and the data from additional trials to come[36] helped mitigate this concern). However, the FDA is still not comfortable with approving antibiotics based only on PK/PD data, as illustrated by the fact that Avycaz was not allowed to get a label for the other indication the sponsor was applying for – aerobic Gram(-) infections, when no adequate treatments are available – because of the complete absence of human data, where just a few case series would have made the voters much more comfortable. Interestingly, the only 'Yes' voter, concerned with this thorny issue, did so to "send a message to pharmaceutical companies" on the basis of such a crushing need and the poorly economically viable business model for the pharmaceutical companies.

The ceftazidime-avibactam combination has then been recently approved by the EMA, as Zavicefta, in April 2016 [252]. It is in line with EMA's guidance from November 2013 which allows for more flexibility in the development of new antibiotics. Contrary to the FDA, the EMA did grant the indication for the treatment of infections due to aerobic Gram(-) organisms in adults with limited treatment options.

[35] Antifungal agents excluded.
[36] The completion of ongoing Phase 3 studies as well as mandatory Phase 4 studies in patients with resistant pathogens are awaited.

Some key elements to understand the issues of antibiotic regulatory standards, such as clinical trials and the patent system, are developed below to provide a background. Then, among the initiatives to improve the system[37], variations to the patent system are further discussed with an original approach.

a. Clinical trials

The case for innovative approval pathways

A discussion around antibiotic clinical trials cannot be undertaken without mentioning the particular characteristic of the approval route of antibacterial drugs with regards to their mechanism of action: rooted in the dissociation, or non-correlation, between the pathogen agent and the phenotypic disease is the explanation for the burden imposed on antibiotic testing by the clinical trials and the case for innovative approval pathways. To understand this point, a brief reminder of the logic of clinical trials is useful. When it comes to the majority of therapeutic testing, for non-infectious diseases, there is usually a very significant (and almost unitary) correlation between agents or pathways at every level of the disease phenotype; and although the target may impact other response signals (whose activation up to the most integrated phenotypic level is responsible for adverse effects), there is one principal and predominant association (univalent association) with the particular cascade leading to the disease. In other words, one target is necessary and sufficient to lead to one disease. This is the most basic and common therapeutic view, although counterexamples exist, such as antineoplasic drugs, where one target or pathway can lead to different oncologic diseases. This main association is incidentally the very prerequisite for drug discovery, since lead compounds are identified by screening their activity on a particular target, and are then entering clinical trials based on the particular, resulting disease. Bacteria and infectious diseases do not abide by this rule though. There is no immediate, unequivocal link between one infectious condition and the responsible bacterium; local skin infections can have several bacterial origins and so do sinusitis (in addition to the possible viral agents), and, conversely, one bacterial strain can be responsible for various infectious expressions at the phenotypic level: one target can cause multiple diseases: *E. coli* can cause urinary tract infections or bacteremia, among other infections. This has an implication for agents against such targets: one antibiotic, targeting one bacterial strain, can, as a consequence, be used for several indications. Were it to be legally allowed and seek approval for these multiple indications, this in turn implies that the drug must undergo as many clinical trials as desired labels. There lies the difficulty in antibiotic clinical testing: multiple trials are required to benefit from a full coverage of one bacterial strain, which implies a bigger development cost profile for drugs that

[37] Extensive reviews on regulatory initivatives can be found at [312], [365], [366].

are already not the most profitable ones and that are being discovered for their activity against one bacterial pathogen, not against one infectious disease.

Is it worth noting that although they share this characteristic with oncologic drugs (which may indeed target one growth factor responsible for the development of various types of cancer, which require multiple trials), antibiotics have additional, specific hurdles in the form of enrolment of patients: whereas cancer patients are diagnosed from the start what type of cancer they have, up sometimes to its molecular characterisation, this is not necessarily the case for patients with infectious diseases where rapid and accurate diagnostic testing to identify not only the bacterium but also its resistance profile is not as widespread. Patients suffering from acute, serious infections may also be acutely ill and require urgent, empiric treatment, precluding them from joining a clinical trial further on. As a result, enrolment and the reach of the critical trial size find themselves impeded. To take an example, it took 18 months for a company developing a novel antibiotic against vancomycin-resistant *Enterococcus* (VRE) to enrol a few 45 patients with the desired infection in the US – whereas the CDC estimates the number of annual VRE cases in the hospital setting (regardless of the infection type) to be about 26,000 [120].

The traditional expectation of a couple of large (usually, more than 700-800 patients per study) Phase 3 studies for approving antibacterial agents that had worked well in the past has now become problematic, with the development of agents against specific or resistant pathogens where enrolling a large pool of patients may be impossible or impractical. With the aim to reconcile and align again the rationale of antibiotic drug discovery with that of approval and clinical trials requirements have some innovative measures from regulatory bodies on both sides of the Atlantic come into existence.

The FDA's trials and tribulations

Underlying this trend is the fact that regulation on and requirements for antibiotic testing has been quite unclear for some time. At least on the FDA side, the guidances for industry had been held up in revision or development for extended periods of time, or reviewed and changed several times. Bearing the cost of the FDA's attitude on the topic is faropenem, a novel oral penem antibiotic, developed by Replidyne. The new drug application was submitted in 2005, including comparative non-inferiority trials that the FDA required for the indications. Yet a few months after accepting the NDA, the company was told that the FDA had changed the requirements *post hoc* and that a demonstration of superiority was now the norm [253]. This uncertainty about the required regulatory elements have put off many industrials from entering such troubled waters, with the most committed to the field postponing their clinical tests to less shady times, and the others simply leaving the area.

This particular problem has been lessened today thanks to newly published guidances (see for instance [254]): between 2010 and 2014, six (final) guidances relating to the development of antibacterial drugs have been issued by the FDA; this is three-quarter of the entire (1977-2014) definitive FDA guidances on antibacterial drugs being issued in that four-year time period [255], and it illustrates well the fact that the FDA has "rebooted" its entire approach to antibiotic development, as announced by the Director of the Center for Drug Evaluation and Research (CDER) Janet Woodcock [256]. Further certainty (and confidence) has been instilled into the field (and industrials) with the fact that no revisions of antibacterial therapies are on the FDA's agenda for 2016 [257]. When it is not the lack of certainty and reliability on the requirements that is a challenge for industrials, it is the other aspect relating to the stringency of the demanding conditions on clinical trials. The American view provides a telling example: the FDA previously had stringent requirements regarding trial sizes, enrolment criteria and statistical thresholds driven to irrational extremes that did not favour the entry of new antibiotics (and in practice were deterring it) [258]. For example, a nosocomial pneumonia trial took nearly five years to enroll about 1,200 patients [259]. The denunciation by stakeholders of conditioning access to market on such out-of-reach requirements led to tentative improvements by the regulatory agencies.

In the draft guidance of July 2013 [254], "Antibacterial therapies for patients with unmet medical need for the treatment of serious bacterial diseases", non-inferiority designs are recognised as possible for the clinical trials of antibiotics targeting unmet needs together with more innovative (statistical) designs such as Bayesian statistics and other approach ensuring more flexibility in the analysis strategies. Historical, as opposed to active controls, are also allowed for these types of drugs under some conditions. Still in its newly-introduced streamlined development programme has the FDA opened the door to trials conducted in "a patient population enriched" where in the context of unmet need can an antibiotic be tested on a trial not limited to a single infection site, a measure deemed acceptable if the appropriate statistical methodology is used (mainly superiority designs). Some flexibility was also introduced in the requirements of safety databases, where smaller patient pools (300 – 400 healthy patients), when submitted with strong preclinical data and mandatory Phase IV studies, may be acceptable.

Another step towards a more flexible regulatory environment, the October 2013 new guidelines for acute bacterial skin and skin-structure infections [260] standardise the use of objective criteria for antibiotic efficacy, proposes a new non-inferiority margin and changes the definition of eligible patients to facilitate enrollment [261].

These legal moves have been supported in practice since their release. For instance, in their December 2014 meeting, the FDA Anti-Infective Drug Advisory Committee (AIDAC) discussed issues related to clinical trial designs for antibiotics indicated for serious infections with limited or no therapeutic options; in face of the unmet need, the Committee was in support of streamlined

development programmes, while such flexibility would not mean lessening their standard on efficacy and safety [262].

A clearer approach set by the EMA

Both the FDA and the EMA have recently recognised the need for an adaptation of their regulatory conditions in order to stimulate antibiotic development and have published updated guidelines, with the EMA having however done it sooner than the FDA, and with fewer back-and-forth considerations that are as many drawbacks for the industry.

- The Adaptive Pathways approach

The EMA is indeed testing a new model of adaptive licensing (or adaptive pathways) which makes use of real-world data: with the aim to improve timely access for patients to new medicines, the drug, initially authorised based on the submitted clinical data and used in a restricted population, can evolve its licensing progressively based on the evidence gathered for access to a broader population. Establishing the strong rationale behind the approach is a tripod of principles revolving around an approval in stages, real-life evidence, and an early involvement of stakeholders. Where the traditional approach would be to test an antibiotic for several organ-specific indications, the alternative approach, recognising the high unmet need, considers using a more restricted indication (Pilot project on adaptive licensing, 2014, [263]). The viability of this approach was further explored with the launch of a pilot project, in March 2014 (ongoing), with the aim to provide, for a selected bunch of qualifying experimental medicine, a framework for the parallel scientific advice from the EMA and health-technology-assessment (HTA) bodies, also involving, where possible, patients groups and provider groups. Illustrating the purpose of the adaptive licensing approach and the regulatory flexibilities that could ensue is the retrospective case study of a novel antibiotic provided in the description of the pilot project (Annex II, [263]); the basis of its adaptive clinical development and licensing is contrasted in Figure 48.

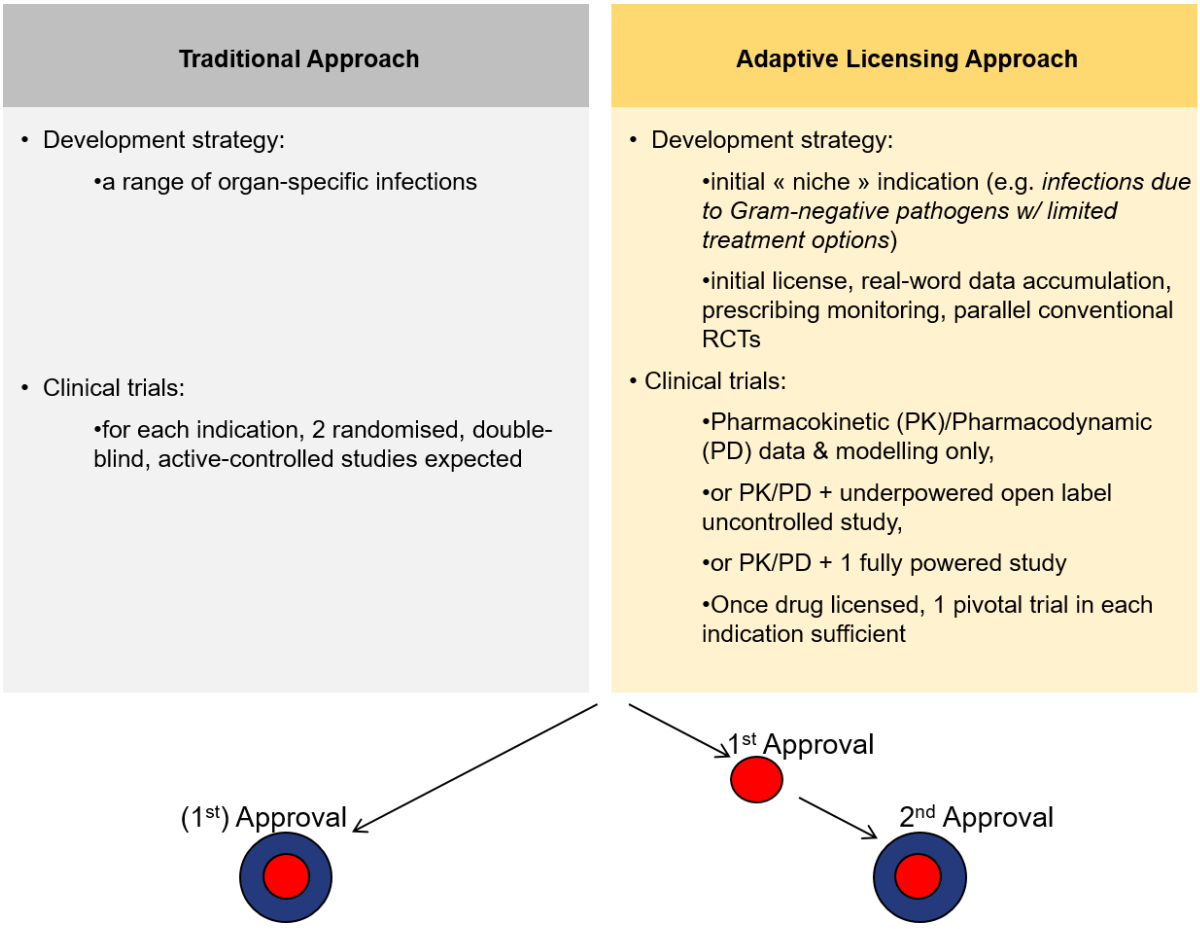


Figure 48. Potential clinical development of a novel antibiotic under the adaptive pathways approach (right) compared to the traditionnal approach (left) (case study). From [263].

▶ Pharmacometrics for clinical development: the PK/PD considerations

As a corollary to this approach is another important measure introduced, the use of pharmacokinetic (PK) and pharmacodynamic (PD) data to establish the clinical proof of the antibiotic's efficacy. The EMA released a specific guideline (draft status) in September 2015 to outline the regulatory expectations on the use of PK/PD in the development of antibacterial drugs [264]. The rationale for the use of such data is based on the accrued reliance and the advances in the field of pharmacometrics. Encompassed in this area are pharmacokinetics, which measure the quantitative relationship between the administered dose, the biological, *in vivo* concentrations, and time, usually through the classic ADME stages (Absorption, Distribution, Metabolism, Excretion), as well as pharmacodynamics, which describe the course and magnitude of the pharmacological effect. Putting both elements together is the PK/PD model, which mathematically describes the relationship between the pharmacokinetics (such as the area under the curve, AUC, a measure of exposure to the drug) and the pharmacological effect (microbiologic measure of bacterial susceptibility, such as MIC). The purpose is to use such models and the PK/PD relationship to identify the dose intervals with the most likely efficacy and least adverse effects, thus removing the need for clinical dose-finding studies. PK/PD analyses would also be helpful in selecting the dose

interval that minimises the selection of resistant mutants, by evaluating MICs for the drug against the targeted organism in the presence of a range of resistance mechanisms, as well as for identifying beta-lactamse inhibitors dose regimens. Additionally, analyses of clinical exposure-response (E-R) relationships can be used to describe the interplay between MICs and PK of the antibiotic, and the outcome of the treatment, with the aim to predict the efficacy endpoint.

Several reasons support the use of pharmacometric considerations: first, drug exposure indexed to MIC is able to predict outcome [265]; second, PK/PD data can be derived from nonclinical studies; and the PK/PD relationships apply equally well in man as in non-clinical model [266]. Therefore, as long as human PK data are available can these models be used to cost-effectively support and enrich the evaluation of limited clinical data towards regulatory approval. They also provide a good balance between data quality/quantity and the unmet clinical need, mainly illustrated by agents targeting resistant pathogens, where very limited clinical development programmes are undertaken. An example of what a PK/PD-based regulatory approval for a new antibacterial drug active against MDR bacteria in the setting of a single randomised clinical trial with other clinical and non-clinical data is provided in Figure 49. While the pre-clinical infection model will generate the PK/PD relationship required for efficacy and demonstrate the impact of resistance mechanisms on the index, the single randomised comparative trial will allow a collection of PK data from patients with wild-type infections to construct the exposure-response relationship; additionally, PK data from patients infected with resistant strains will be collected during the smaller, non-comparative trial, allowing the integration of results across the entire development programme.

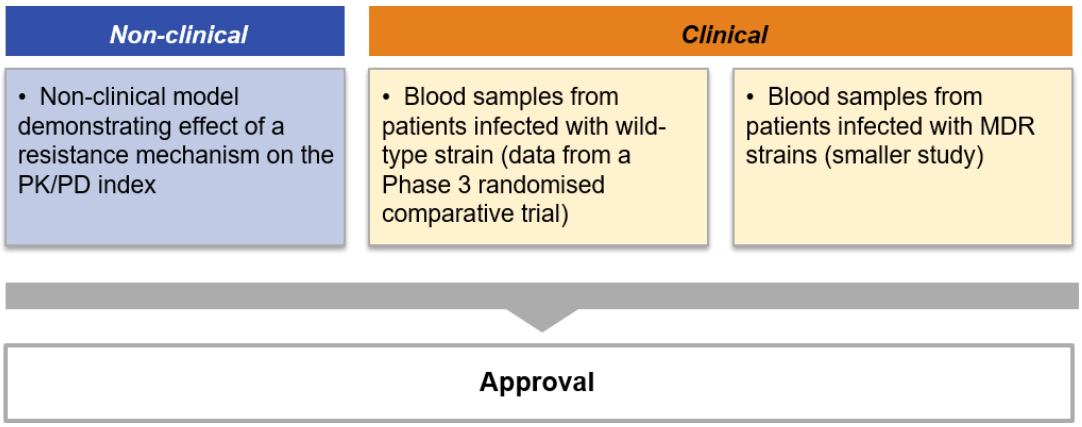


Figure 49. The "pharmacologically-based package": example of PK/PD analyses in support of the approval of a novel antibiotic active against resistant organisms. Adapted from [266].

▶ Introducing the Pathogen-Specific approval

To make the clinical trials less demanding and burdensome, the EMA also approved a new guideline on the evaluation of anti-bacterial treatments with the innovative measure of pathogen-specific

approval (further detailed in the next paragraph; Guideline of 2011, [267], and its 2013 addendum, [268]). The guideline recognises that clinical data against specific pathogens may be gathered in the clinical trials that enroll patients regardless of the body site(s) affected, with the potential to relieve some of statistical burden imposed on the trials. Such efficacy data would lead to a pathogen-specific indication if the drug has demonstrated efficacy against the organism causing infections at a range of body sites. While also revisiting issues on patient selection criteria, primary endpoints, conditions on the use of superiority or non-inferiority designs and margins, the EMA's aim is to be practical, transparent and conducive to antibiotic development.

The pathogen-specific approval pathway: a measure with substantial economic implications

It may be that an important answer to the challenge of multiplicity and the recruitment difficulty of antibiotic clinical trials lie with the pathogen-specific pathway. For one thing, there is already precedent for such adaptive, organism-specific clinical trials, for instance with invasive fungal infections [269]. For another thing, the diminution in development costs when changing the conventional approval scenario[38] for the pathogen-specific scenario[39] would be substantial: with improvements in phase success probability and duration, the average costs are reduced from $2,190 million to $752 million (see Figure 50). Whereas both projects are adjusted for early R&D failures and assume an 11% annual cost of capital, the near two-thirds reduction in costs is explained by the time from preclinical tests to approval, a condensed 10.5 years if the regulatory pathway is adapted. These figures are not definitive and could be challenged and subject to many adjustments[40], but they illustrate the point that providing regulatory solutions can improve the economic model of antibiotics quite a lot. Additionally, the pathogen-specific scenario would also reduce the number of patients to be enrolled [270].

[38] Scenarii include two standard Phase III trials of the bacterial strain in a targeted unique infection site.
[39] Scenario includes small prospective studies and descriptive data for the bacterial strain in an array of standard infections.
[40] The final figures are extremely sensitive to the initial hypotheses, e.g. failure rate assumptions, phase success probabilities, novelty of drug candidate. Just to illustrate this claim and provide some perspective to the reader, an independent research piece provides drug attrition rates, from preclinical to approval steps, of 40%; 75%; 48%; 64%; and 90%, sequentially [360], while the commercial organisation IMS has their own data on observed rates of 9.3%; 33%; 75%; 85.7%; 75% [361].

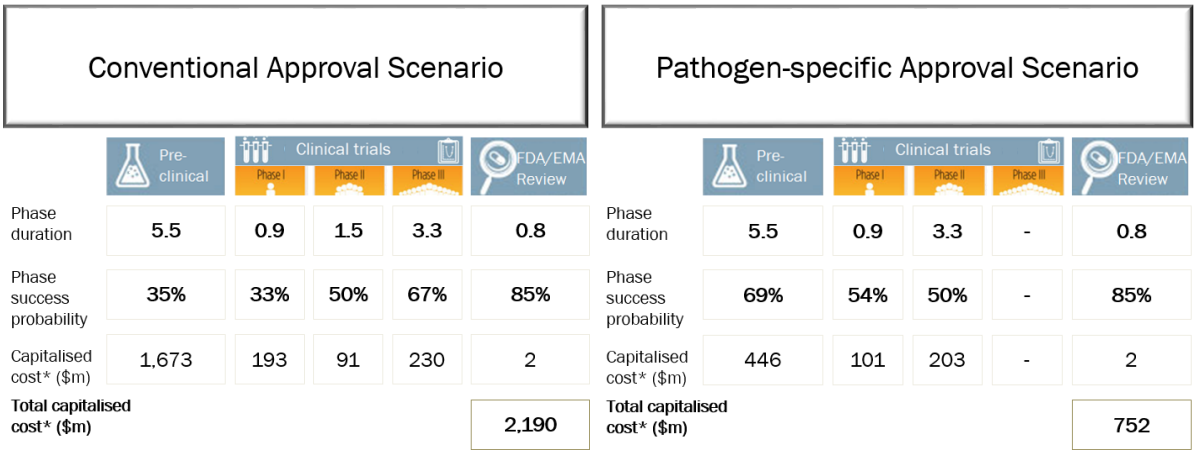


Figure 50. Comparison of the traditional approval and pathogen-specific approval pathways.: the capitalised costs and total costs include R&D projects that failed. Note: rather than undertaking phase II studies and phase III studies, the pathogen-specific scenario conducts 'adapted' studies of intermediary size, the figures were classified for convenience in the 'phase II' studies column. Adapted from [271].*

This regulatory improvement, already passed into regulation by the EMA in its 2011 guideline, will mostly impact the first part of the challenge, the multiplicity issue, by trading a collection of trials in several infectious conditions for one trial with the common responsible pathogenic bacterium. The enrolment issue may remain, though, as the previous solution will indeed permit to mechanically increase the pool of available patients for trials but contributes very little to the identification problem. Patient enrolment is either delayed until the results of a positive laboratory diagnosis, or impeded if the patient is recruited but then found not to be in the target group for the antibiotic. For example, only one in four people of the population in a trial targeting *P. aeruginosa* actually have the right causative bacterium ; having to screen and register at least 800 people in order to run a trial with 200 truly eligible patients drives up the costs and difficulty of the trials [272]. Additionally, in a trial comparing combination therapy to monotherapy for the treatment of suspected ventilator-associated pneumonia, 18% of the 739 patients did not have any infection at all (no organism grown in enrollment specimens; [273]). Hence, the ability to identify the exact cause of a patient's infection, which translate into the use of rapid, accurate and specific diagnostics, is the best-suited response to this problem [274], and whether it is through the companies' own understanding that better diagnostics will help them with the bottleneck issue of trial enrolment, or through regulatory enforcement and requirements by the responsible agencies, these tools are meant to be essential components of the clinical trials for antibacterial drugs[41].

The picture on antibiotics clinical trials would not be fair and complete if stopped with these arguments; it is also useful to remind here that, because of the acute nature of the diseases they are

[41] See note 13 for reviews on the need for and the strategy behind antibacterial diagnostics.

targeting, and the great efficacy of the agents, the efficacy trials (Phases 2b and 3) are shorter than for other conditions, which in turn means that the costs may be lessened; indeed, antibiotics register among the fastest clinical development times (87 months) of any therapeutic class [275].

b. Patents

Setting the stage: the traditional debate and the current practice on patent extensions

Secondly, the aspect of legal protection has given rise to impassioned debates and controversial proposals. On one side are some economic experts arguing that extended patent protection periods or wild-card systems (see below), with the extra profits that they will generate, are needed to compensate the lower financial viability of antibiotics relative to other drugs. The counter-arguments that immediately arise include the social drawback from a *de facto* delayed apparition of the generic equivalent[42]. Regardless of any stance is the view that such measures would be technically inefficient anyway. Taking a pro-industry side in this debate have the US gone the extra mile with a bold new incentive combining regulatory and intellectual property advantages. Under the Title VIII of Food and Drug Administration Safety and Innovation Act (FDASIA), entitled Generating Antibiotic Incentives Now (GAIN) Act (October 2012), a new antibacterial molecular entity that addresses an urgent threat may be granted the QIDP designation, or Qualified Infectious Disease Product. Among the benefits unlocked by this designation are a five-year extension of market exclusivity (with an additional six months if the sponsor develops an accompanying diagnostic test) as well as a Fast Track and Priority Review approval process [276]. The first antibiotic approved under this designation was Dalvance in May 2014, and all the antibiotics approved since in the US (6 to date) have been designed QIDP products [277]. This can be visualised in Figure 47 with the peak in 2014 approvals that follows the introduction of the measure in 2012. Regulatory moves from the FDA are considered the causal element for this shift: what was initially designed as an end reward for innovative drugs, as for bedaquiline (treatment of multiresistant tuberculosis) in 2012 (rewarding the output), is now used more proactively as part of a macro incentive scheme to set in motion new antibiotic development (incentivising the development), and the challenge is now to manage to make a distinctive use of this measure so that it can keep its efficacy. Indeed, if this measure has incentivised the development of new molecular entities, it has not resulted in the production of new therapeutic classes, as all the latest antibiotics approved are variations of existing scaffolds. Whether the QIDP measure has had any other positive impacts is not clear: there is no evidence that the earmarked drugsaddress the issue of resistance specifically, are more effective than previous antibiotics or meet unmet medical needs: indeed, half

[42] The well-known advantages and drawbacks of variations to patent law have already been assessed and thoroughly described in other pieces of work and are not to be restated here (see for instance [362]).

the drugs were approved for the same indication (acute bacterial skin and skin structure infection). There is little doubt that teixobactin could qualify for this designation as well, easing its going through the regulatory process and potentially increasing its profitability down the line.

Extended patent protection periods can also take the form of a restoration of the patent time foregone during the regulatory review times. With a mean time as long as 18 months for the FDA (traditional review pathway) [120], the patent holder is losing more than 5% (one in twenty years) of their IP protection. Knowing that the longer the patent duration, the higher the marginal profitability[43], and considering the potential profits that can generate an extra 6 months of patent protection, this is far from being an insignificant reward, although not a sufficient draw if used alone.

Some would go even further, up to a case for "infinite-term patents" on antibiotics where the guaranteed high price for the entire lifetime of the drug would be a required condition for industrials to accept to rationalise the usage of antibiotics and to hold them in reserve [1]. Without a limit on patents, there is less incentive for waste: the time-limited nature of the property right is encouraging the maximisation of sales under the protected period rather than long-term resource management [242]. The dilemma that antibiotics patent holders face is even more pronounced compared to that of other therapeutic classes; not only is every manufacturer of a protected drug facing a difficult choice between aggressive marketing and rational usage, but the specific issue of resistance related to antibiotic consumption makes the case for rational use even stronger with the necessary conservation measures to curtail resistance. Because of the biological characteristics of resistance, waste (and sales-maximising attitudes of firms) is not only detrimental to their patented molecule, but also to the existing and subsequent drugs in the same antibiotic family (cross-resistance).

Further discussing the patent extension measure: a new perspective introducing the public health interest

The discussion on patent extensions could benefit from another perspective: the public health interest. This measure does not seem so far-fetched if one considers that genericisation of antibiotics is a double-edged sword rather than a blessing, and that there may be positive implications of patent extensions for public health. The cheaper development of identical molecules by asset-light generic manufacturers, or genericisation, brings to market a much less expensive version of the initial drug. The rationale is to make drugs affordable to a larger pool of patients, since, according to basic economic concepts, from a lower price will ensue a larger demand (therefore a better access).

[43] This is true for traditional classes of drugs. When it comes to antibiotics, this statement is likely to be mitigated, although it is true that use is mostly deterred at the beginning of the marketing period, and, as resistance to first-line agents develop later on, usage usually augments.

Indeed, it has been demonstrated in practice that genericisation is the most effective way to lower medicine prices [278][44].

Introducing generic competition has the most positive consequences for public goods with positive externalities, items that have a greater value to society as a whole compared to the benefits they bring to the individual (see definitions in Section 1 of this Part). Yet, in the case of compounds with negative externalities, this measure has received much less attention and is likely to be detrimental. Indeed, basic economic principles command that the price of goods with negative externalities be higher, in order to deter their use and the negative consequences for society. The tangled case for antibiotics makes the patent discussion a very tricky one. Both aspects of externalities are present. But it is contrary to antibiotic rational usage and stewardship measures to go down a route of improved access and facilitated procurement; instead, and for all the reasons described so far leading to the apparition of resistance are antibiotics better off "shelved" than spread. A patent on an antibiotic creates a monopoly that helps mitigate overuse. It is hence not evident that, for the particular case of antibiotics, generic apparition (and a free market) results in an increased benefit to society compared to patented situations (and monopolies); one has to weigh up the apparent benefit (the lower price that the individual will face) with the indirect costs (cost of negative externalities to society due to a greater consumption). Generic access to cheap (or even free) antibiotics is not entirely beneficial for the public health, as it drives resistance [279], [280].

Hence, the case for extending patent protection holds if it prevents the usual rise in volumes following the patent end. But is this very premiss true? An increase in volumes is what economic theory predicts, and what is observed in general with most drugs, yet with antibiotics the evidence is inconclusive as for the trend after IP rights expiry. A recent IMS survey gathered information on the impact of generic entry for two types of antibiotics (a carbapenem and a fluoroquinolone) in six high-income healthcare systems. Based on sales data, there was no general conclusion or consistent impact of generic introduction detected by the research. While in some countries usage diminished, in others it increased. In the former case, there would be no need for intervention; in the latter, there would. Even with the inconclusive results, a measure that would diminish volume in this dual context would only be beneficial.

Actually, as the trends in antibiotic usage after patent expiry does not seem to correlate with, or be impacted by, the end of the patent and the availability of cheaper equivalents, they might rather reflect the more general driver of antibiotic prescription, which lies with their very effectiveness. As antibiotics progress in their marketed life and are used over time, they decrease in clinical effectiveness. This means that while the financial effect of extended protection is small to the

[44] This effect happens not only through the lower-priced generic drugs, but also through a decrease in the price of the originator's patented product too.

pharmaceutical industry (this measure could not be used as an effective incentive to develop new antibiotics, and their insufficient incentivisation characteristic is further acknowledged by Sertkaya et al in their analysis of incentives [281]), it is also likely to not weigh much on society either, as prescription would regardless be self-limited in the view of obsolescence, resistance, competition from follow-on drugs.

The measure could, however, through the longer drug monopoly that they grant, be useful in that they entice the industrial to consider the longer-run life cycle of its antibiotic, and in order to keep it effective for a longer time, the resulting conservation measures will be aimed at delaying the emergence of resistance[45]. This behaviour is rooted in economics theory (the limited-term monopoly affects behaviour in the opposite direction of an unlimited monopoly, with the incentive of the profit-maximiser seller to cram most of their sales into the monopoly period rather than stretching consumption over time) and observed in practice (Pfizer, the seller of linezolid, whose sales have escalated since approval in 2000, even received a warning letter from the FDA for its misleading promotion of Zyvox [282], reflecting strategies to enhance sales and marketing).

Whether the delay in generic apparition actually hinders access is probably unlikely. Outterson et al [283] state that high drug prices will be detrimental since they will translate into denied financial access for patients to needed therapy. However, it is argued here that this is view does not hold when taking into account first the specificities of pharmaceuticals economics in general, and of antibiotics economics in particular. It is known for a fact that, in high-income countries with national health insurance coverage, patients are relatively price-insensitive when it comes to drugs, as they have negligible out-of-pocket cost on medicine and hence do not tend to vary their consumption (demand) with regards to price (in economic terms, demand is inelastic) [284], [285]. Another fact in health economics is that demand is not actually resulting from patients' (consumers') preferences as in basic economic theory; a third-party, the physician, is responsible for making the consumption decision on behalf of the patient, and therefore their (not the patient's) preferences drive demand (this is referred as to a principal-agent relationship), and unless there are major market inefficiencies, physicians' preferences are not driven by price but by rational considerations inculcated by their medical education (although in practice physicians can be irrational agents) [286].

Added to these general features that hold for antibiotics too [287] are specific antibiotic features: again, thanks to their medical knowledge will physicians be aware of the externalities associated with antibiotics' usage and volume, and hence be even more entrenched in their anti-infective

[45] Outterson counterbalances this view with the conjecture that publicly traded companies with quarterly earnings target would still have an incentive to boost their present sales [242].

prescriptions, strongly advocating (proper) usage when needed and strongly forbidding it when inappropriate.

All these arguments converge towards the conclusion that price would not deter access under these conditions[46]. Whether this still holds in practice needs further researching and quantitative modelisation; especially in developing countries where the issue is particularly challenging (most of the above premisses do not hold). Some authors abound in this direction, stating that the lower treatment rates associated with a prolongation of the patent is socially desirable when infection levels are relatively low and antibiotic efficacy levels are high [288]. An extension of patent life may be a good measure, if it leads to a more reasoned antibiotic usage, not if it harms access, and this is the main pitfall that has to be prevented in this regulatory measure (Table 7). By putting a strain on antibiotic usage, the measure could provide a good solution to the issue of negative externalities arising with over- or misuse; a higher price will create the necessary wariness.

	Positive impacts	**Negative impacts**	**Neutral/Questionable impacts**
Pharmaceutical industry	• Incentive to take efficient, longer lifecycle management measures: conservation measures	• For time-limited patents: near patent expiry, incentive to maximise sales • Profit still linked to price and volume; no delinkage strategy • Postponing / removing threat of patent expiry: no more driver to develop new drugs / negative impact on antibiotic R&D?	• Financial rewards likely to not be substantial (will neither incentivise nor deter antibiotic R&D), since additional patented years may not generate additional sales because of resistance
Patients and society	• Limited usage: control / limited spread of negative externalities	• Delay in genericisation: hinder access?	• Volumes not impacted by / correlated to patent expiry?

Table 7. Taking the discussion further: benefits and drawbacks of patent extensions.

This is but one solution; are there others that might be more useful to address the issue of negative externalities? Since negative externalities are essentially linked to overuse and misuse, rapid diagnostic tests could also be a solution. According to estimates from Shapiro et al [289], out of the annual 40 million people who get antibiotics for respiratory conditions in the US, 67.5% get

[46] As an aside, price does not burden patients but health insurance systems. The questions then turn into the normative concern of whether the extra social costs generated, if any, should be taken up by these public sector actors, and, in a more practical setting, of what would be the resulting actions taken by these organisations to mitigate their extra costs. Outterson argues that if the consequences are to be passed onto employees and result in managed care techniques, then it would be easier to implement them directly without bothering with extended patents [242].

antibiotics for a questionable indication (displaying conditions for which antibiotics are rarely prescribed). Furthermore, taxes on inappropriate use, the economic's scholar answer to negative externalities, can also be considered; yet determining whether the use was adapted or not is quite impossible with empiric therapy and will require, once again, the availability of supportive diagnostic agents. The consequence that taxes bring – by factoring in, or internalising, the external costs consequent to use of antibiotics – is the same as for the patent extension measure: in both cases, the higher price, consequent to the tax or the patented status, leads to a decreased usage. There is, however, no solution to the "extended" negative externalities issue, the passing on of resistance genes to other strains, as no barrier can prevent promiscuity and the transmission between species.

To conclude on this argument, if it was not for the deterred access ("deadweight loss") associated with monopolisation[47], patents (and their extensions), by stretching consumption out over a longer period, can provide a comprehensive solution to the problem of antibiotics. Whether this trade-off is attractive enough is down to regulatory and public health policy stakeholders, urged to very carefully reconsider the original rationale of genericisation, a measure designed for individual and chronic therapies, in the light of antibiotics' unique specificities, and to weigh up its benefits against the costs.

c. Other examples of possible routes for regulatory adaptations

The evolutions that have taken place so far and been implemented, whether being non-specific or targeting the antibiotic industry itself, are summarised in Table 8 and explored in the first part of this section. There are still other legal and regulatory incentives that are being proposed by scholars, which are analysed further down in this section.

Existing measures to benefit antibiotic development

Gathering a couple of non-specific, purely regulatory incentives are global solutions such as the Orphan Drug designation, and regional tools such as the adaptive pathways (EMA; see above) or the fast-track designation (FDA).

First, and going further to the GAIN's five-year market exclusivity extension are proposals that would grant market exclusivity conditions and IP incentives similar to those in place for orphan

[47] A patent creates a legal monopoly on its product. In economic theory, when a monopolistic situation arises, the producer, whose aim is to maximise profits, does so by diminishing supply and increasing prices, compared to the equilibrium in perfectly competitive markets. As a consequence, some of the consumers facing higher prices cannot afford the goods anymore, and their loss is called the social loss or deadweight loss [363]. This deadweight loss is however a trade-off for investment in innovation.

Challenge ('Before')	Proposal ('After')
Non-specific challenges for important medical needs	*Orphan Drug designation; Breakthrough Therapy designation, Accelerated Approval, Priority Review, Fast Track designation (FDA); Adaptive pathways (EMA)*
Demanding technical requirements	Adapted and wider NI margin (GAIN Act°) Possibility to use historical controls (FDA)
Problematic trial size and enrolment	Definition of eligible patients relaxed (GAIN Act); pathogen-specific approval pathway° (EMA)
Poor return-on-investment	Additional five years of market exclusivity (GAIN Act)
Case where human efficacy trials would be unethical/unfeasible*	Approval without any human efficacy trials*

*Table 8. Evolutions in the regulatory challenges of the antibiotic industry. In italic are cases that are non specific to antibiotic regulation yet can apply to antibiotics. °: detailed in Section 2.C.a; *: this case is very rare and has been used in practice only once, in 2012, for the approval of raxibacumab, a monoclonal antibody for the treatment of inhalation anthrax [290].*

drugs. If it meets a number of conditions (regarding the seriousness, prevalence and lack of satisfactory treatment options for the condition targeted), a drug can qualify for orphan designation. This status unlocks several incentives, helping with clinical development (in the form of protocol assistance), market and competition (10 years of market exclusivity, under some conditions) and financila considerations (fee reductions for marketing authorisation applications, various national and EU financila incentives in the form of grants and tax reductions) [291]. In the US, under the Orphan Drug Act, the scope of orphan-drug exclusive approval is of seven years, and further agreements such as tax incentives or the availability of grants are also in place [292]. There is general agreement that these measures have had positive effects to encourage the development and marketing of drugs to treat orphan conditions [293], [294]: for instance, the effective patent and market exclusivity life of orphan NMEs was increased by an average of 0.8 years compared to non-orphan NMEs in the US [295]. It is such a positive impact that advocates of similar designations transposed to antibiotics want to see reproduced.

Furthermore, other non-specific regulatory proposals include the US Breakthrough Therapy designation, a status under which qualifying drugs may undergo a significantly less burdensome approval (it can be based on surrogate endpoints or on pharmacodynamic biomarkers, if they are predictive enough of the likely efficacy in practice, or on an improved safety profile) with intensive guidance on drug development. The Accelerated Approval regulation takes up the principle of

approving drugs for serious conditions based on a surrogate or intermediate clinical endpoint as well. Priority reviews organise resources to direct them to the evaluation of applications of drugs representing significant clinical advantages. Under the Fast Track status, granted for drugs targeting unmet needs or whose improvement over current therapies is significant, the FDA commits to more frequent meetings and written communication and grants the Accelerated Approval and Priority Review mechanisms if criteria are met [296].

At the pilot stage in its non-specific form, the adaptive pathways approach introduced by the EMA could also work well if applied to antibiotics. The rationale for the evaluation and licensing of antibiotics based on a specific pathogen rather than a specific (infectious) disease is quite strong, as highlighted above (Section 2.C.a), and the rewards non negligible. Yet there are further implications to such an idea, with for instance spillovers on the regulation of diagnostics. Any narrower-spectrum drug will require for its appropriate use the accurate identification of the sensible pathogen, and therefore the need for diagnostics able to rapidly and specifically identify the bacterial strain. Diagnostics R&D appears to be up to twice less expensive, with the potential downside of a less profitable market because of uncertainties around adoption and reimbursement [271]. In the context of diagnostics development for techniques and processes that are likely to be a more and more necessary parallel product package in the antibiotic market, the question of diagnostics regulation needs to be addressed as well. The current system is made of a more innovation-supportive EU pathway and a demanding US system that can be so problematic as to deter an American commercialisation [297], [270]. Bringing more certainty about the future market of bacterial diagnostics, as with antimicrobial drugs, is essential to ensure company investment. It could also have the positive effect of improving the business model of narrow-spectrum antibiotics, by mimicking the specialised oncologic drug – companion diagnostic model. Not only would such a model be immune to overuse, as use would be conditioned upon the rational diagnostic tool, but it would also permit to further the development of diagnostics, deliver narrow-spectrum agents (which are arguably more useful than broad-spectrum ones, as explained in Part 2), and improve the profitability, as a premium pricing for the antibiotics, like for specialty drugs, would be conceivable. Whether this business model could indeed solve as many problems at once in practice is not developed here but would actually be worth considering [298].

Going further: proposals for more regulatory adaptation

A proposal coming from IDSA and gaining much support from the industry is the Limited Population Antibacterial Approval pathway (LPAD) [269]. To incentivise the development of antibiotics to treat severe infections caused by very resistant bacteria (XDR/PDR organisms), clinical trials could be shortened and rendered less expensive by the conduct of studies in substantially smaller populations (IDSA supports a size of the pivotal trial as small as 30 to 100

patients). The drug would then be granted a narrow and specific indication. The society advocates that such a greater degree of uncertainty could be tolerated in view of the seriousness and lack of treatment options for patients infected with the most resistant organisms.

Some publications are further discussing the potential approaches to improved antibiotic study design options, such as with orphan-like regulations, tiered frameworks [299], nested superiority/non-inferiority trial design [269] or measures from other therapeutic segments [258], [300].

More controversial measures have been put forward to improve the intellectual property protection, such as wild-card patent extensions. This mechanism stipulates that for a company that is granted approval for a priority antibacterial drug, an extension of the market exclusivity period is made possible on another approved drug in the company's portfolio. Because this measure is unlikely to find favour with policymakers, additional compulsory conditions can decorate this proposal, such as a necessary commitment to antibiotic R&D by ways of a re-investment of the generated profits. While being undoubtedly associated with large societal costs (a "hidden tax" on conditions treated by the industry's best-selling products, those which are more likely to be the beneficiaries of the wildcard, such as cardiovascular diseases or musculoskeletal conditions), this measure has been argued to also be of uncertain economic benefit to the manufacturer [301]: a quick numerical calculation showed that, for a one-year extension of the patent on Cymbalta, based on 2012 sales and supposing that 50% of the extra revenues are profits, patients pay about $4 billion for the drug while the company gathers at best $1.2 billion as a reward to incentivise new antibiotic development [302]. A core problem with such wildcard patents also lies with the signal they send: by delinking reward from innovation, they weaken the rationale and the very purpose of IP rights, and furthermore their value [283]. This measure is however considered by the US Congress, which has proposed it as a reward for companies developing agents against bioterrorism threats under the BioShield II Act [303]. The measure is not yet enacted but has already been subject to controversies [304].

Another regulatory measure that could be applied to antibiotic drugs, the system of transferable priority review vouchers in the European [305] and US regulatory systems [306] was originally developed to foster R&D in neglected diseases. The idea of offering a priority review voucher to a drug company that develops a drug for an unmet need, a voucher that can then be used to facilitate the approval process of any other drug, could be applied to antibiotic in lieu of drugs for neglected diseases. Yet this measure has been controversial: since the voucher is only interested when used for drugs that are non-priority ones, it will diminish the value of the priority review system, which will rush into an approval decision for a drug whose benefit/risk profile or added value may be low. On the other hand, the advantage of this mechanism, as for with wild-card patent extensions, lies in

the fact that they are off-budget subsidies for the government; they do not require actual money to change hands and the fact that there are no explicit figures labelling the cost of the mechanism, leaving the estimated value to the sole appreciation of the beneficiary or applicant, is also a positive point to the public sector. This measure can be used as a component of the proposed Antibiotic Corporate Bond model described in the next section and its interest is discussed in some more details further on.

d. 2016: where do we stand on a regulatory perspective?The challenges addressed and the ones that remain

This comprehensive bunch of regulatory adaptations, whether already enacted (GAIN Act, EMA's guidelines of 2011 and 2013) or still at a pilot/draft stage but about to progress (Adaptive pathways in Europe, draft guidance of July 2013 in the US), currently ensure that the development of antibiotics is more rapid and cost-effective. When attempting to quantify that the impact, it has been estimated that some feasible measures such as the pathogen-specific pathway could more than halved the development cost for a narrow-spectrum antibiotic with a new mechanism of action [271]. Since this is not the main topic of this thesis, it has not been emphasised enough how important the contribution of diagnostics to curbing resistance can be, yet support for their development, including public sector support, is an essential measure that could also make drug R&D much less expensive. Additional public funding for antibiotic R&D is advocated by many researchers and stakeholders while, with the view that private research is more efficient than the publicly-funded one, modifications to the patent system have much potential in addition to addressing some specific public-good challenges of antibiotics. Noting down the point of view of the industry, studies have concluded that the widespread belief is that regulation is no longer a constraining factor [270], [271]. What is of further uncertainty to the industrials is the commercial factor: how will they make money with a newly developed antibiotic?

D. ECONOMIC

After the scientific and the regulatory components of the ecosystem surrounding the antibiotic industry, this section is interested in the interactions between economic dynamics and antibiotic R&D decision making by companies.

Underlying the current situation with regards to antibiotic development and explaining the declining interest over the past decades in this therapeutic field are the particular economic factors (such as development costs, profitability profile or sales outlook) developed below. It has been claimed in many publications that the economics of antibiotics are not the most attractive to pharmaceutical companies, compared to other therapeutic classes. Why is it the case? This paragraph bridges the

gap between the characteristics that make antibiotics stand out, as highlighted in the first section, and the resulting economic consequences that impact their return on investment. Overall, the implication of them being 'public goods' and having externalities (both positive and negative) makes a case for government intervention and are yet another reason to care about antibiotics development.

a. Indices of profitability – and their application to antibiotics (or: the economic justification of market failure)

Compared with other therapeutic areas, the antibiotic segment is a less profitable market: it represents only 5% of the overall pharmaceutical market, and the annual growth, that was on average 4% between 2005 and 2010[48], is forecasted at only 1.4% until 2020 [307], [237]. The size of the market for antibiotics[49] was estimated through the 2009 global sales to weigh about $42 billion. To illustrate the lower profitability, the best-selling antibiotic in 2003 amounted in about $2bn in sales, whereas a statin blockbuster made more than $9bn [308]. Among the economic factors that explain this low commercial attractiveness are their modes of action: the fact that they act well (they are curative agents, after all) and fast (they treat acute conditions, limiting treatment durations to prevent resistance development) means that the whole point of antibiotic treatment is to be brief (in practice, as brief as possible). Because infections are acute diseases and in order to prevent resistance, the short-term prescriptions and the tendency to reserve new antibiotics for severe cases limit the potential for appreciable profits, to the point sometimes that initial R&D investments are not recouped. Indeed, the model that pharmaceutical companies use to obtain a return on their investment, broadly speaking, is to increase revenue, by either increasing volume or prices, according to the formula equating revenue to volumes time price. But higher volumes of antibiotics will lead to the acceleration of resistance; besides, stewardship measures are in place to ensure that new antibiotics are used only once older ones have lost efficacy. The second element of the equation, price, is not a viable option either, considering the impact on public health budgets [309]. Moreover, by the time an antibiotic moves to the first line of treatment, it is near the end of its patent life, meaning that the forthcoming generic competition does not make it worth it. These elements explain the unattractive commercial outlook, yet the commercial uncertainty is further worsened by the difficulty of getting reliable estimates for the number of cases or the regulatory decisions from HTA bodies [271].

[48] The 4-year compounded growth rate was even stronger at 10% in 2003 [367].
[49] This category represented about 46% of total sales of anti-infective compounds (which includ antiviral drugs and vaccines). It is worth noting the shift among anti-infective subcategories, in terms of distribution of sales: antibacterials do not weigh the most anymore, whereas they representd 62% of the anti-infective market in 2003 [367].

When it comes to the actual decision making on whether to invest in antibiotics or not, sponsors also base their decision on revenue simulations, as they employ a tool that pools future revenue across time and weights them according to the time value of money (uncertainty of future revenues). This tool is called the Net Present Value (NPV), which is formally expressed as the sum of the discounted future cash flows. The higher the NPV, the more profitable the project, and a positive NPV is the minimum threshold for investment (or the minimum result to break even, while the more practical threshold or tipping point that companies use as a benchmark is in the region of $100 million [281]). Although the trends in cash flow are the same for all drugs, with the first years spent investing in R&D and clinical trials, and the years from marketing authorization generating revenue, the scale and extent of these numbers are different for antibiotics. Estimates of NPV vary from ($4.5m) to $37.4m, depending on the indication; negative estimates were confirmed by other reports [310], [311]. These figures contrast with two set of numbers: on the one hand, the NPV for other therapeutic classes such as musculo-skeletal, CNS or oncology, that range from 8 to 30 times the higher end of the antibiotics NPV [227]. On the other hand, the social value for antibiotics, that ranges from $487m to $1.2bn, illustrating once again the difference in social and private benefits [281]. As explained at the beginning of this paragraph, the specifics of antibiotics, namely their low volume, due to a reserved use, their relatively low prices and, in the previous category, their clinical requirements translating into expensive trials, as enrolling the sufficient number of patients with rare or site-specific pathogens requires a timely, accurate diagnostic difficult to realise when faced with urgent conditions, are all reasons behind a lower NPV. Thus, investing in antibiotic R&D is not financially sound with the way the current business model is framed.

b. Documented economic proposals to alleviate unattractive economics

In addition to the refinement of current legislation and regulation would economic incentives such as increased prices, government-backed grants or other funding mechanisms be welcome in the area of antibiotic development. These actions have been plead for by many societies and institutions, such as the Infectious Diseases Society of America (IDSA) [120] or the European observatory on Health Systems and Policies/LSE [312] and are here just briefly reviewed[50], in order to provide an insightful background before the exposé of the core proposal of this thesis, two innovative economic solutions to tackle the economics of antibiotics and their resistance.

The financial solutions are so plentiful that the area could use a classification, and it is common to divide them according to the point in development at which the incentive is granted. Pull incentives are outcome-based rewards: they pay for the outputs of R&D, whereas push incentives are early-

[50] More thorough reviews can for instance be found in [302], [139], [364], [368].

research subsidies to lower the costs of R&D, and both could provide solutions to improve the economics of antibiotics [312].

Because they fund R&D efforts *ex ante*, irrespective of the outcome, push incentives are the most commonly used [313]; they include for example direct funding by the US National Institutes of Health (NIH) or the EU's Innovative Medicines Initiative (IMI), or other incentives such as research collaborations. Funding options are among the most common financial solutions, because public subsidies are designed to encourage products that are undersupplied in traditional competitive markets. The Review on antimicrobial resistance, for instance, is a strong proponent of a dedicated fund fed by the big pharmaceutical companies themselves and offers practical solutions to the issues of leadership, focus and accountability [182]. Among government and public sector funding, the Innovative Medicines Initiative is a European joint undertaking between the EU and the European Pharmaceutical Industry Association (EFPIA) created to provide funding for antibacterial drug discovery and development efforts[51]. However, the common feature of these financial solutions is that they do not generally have a sustainable funding model, putting them at political risk and viewed as a possibly unpredictable cash granting system. An innovative idea drawn from the insurance industry offers to address this sustainability issue by deriving a funding pot from insurance premiums paid in the context of an antibiotic insurance cover [314]. Tax incentives, another part of the pool of "push" solution, are however scarcely ever used. Perhaps the most basic incentive to correct market failure through government intervention, a well-designed tax structure could, however, address the problems ensuing from antibiotics being public goods and having externalities. In theory, a tax can be set up to correct market prices so that the private costs match the social costs of the consumption[52]. In the context of antibiotics, raising the price by a tax increment induces patients to take full account of the entire costs associated with the use of the antibiotic drug, although this mechanism works optimally when demand is sensitive to price, or is elastic, which is not always the case in the context of health economics (see an explanation in the discussion of patents). Hybrids between legal and economic incentives are proposals such as tax breaks for antibiotic R&D or measured liability protections, where claiming tax credits, or benefiting from a zero capital gains tax rate on earmarked bonds have been offered as potential statutory incentives.

On the other hand, pull incentives reward R&D efforts *ex post* if the outcomes correspond to a public health benefit, such as with prizes or advance market commitment. One of the initial measures, the guarantee of an end-market sets a floor on the market when the state is the only

[51] Their lead "New Drugs for Bad Bugs" programme was set up as a response to the rising threats of antibiotic resistance and started in 2012; it is now supporting seven projects [357]..
[52] This approach is also called a Pigouvian tax, after the economist, Arthur C. Pigou, who proposed using taxes (or subsidies) to discourage (or encourage) actions that cause negative (or positive) externalities.

purchaser, and has been proposed for specific antibacterial products with the US government's Project Bioshield Act. With the aim of enriching the therapeutic arsenal and solutions against bioterrorist threats (two-thirds of which are of bacterial origin: anthrax, botulism, tularemia and plague) has this new legislation been introduced, shortly after the 2001 anthrax attacks, earmarking $5.6 billion to fund private-sector R&D of relevant new agents, drugs or vaccines, as well as diagnostics with the federal government serving as purchaser [315][53]. This measure, also called advanced purchase commitment, can be controversial in its valuation of prices or purchasing sums, since the traditional market mechanisms are not at play and efficiency is not guaranteed. Researchers have estimated that the lump sum the government would need to pay over a decade to bring to market 15 new antibiotics (of various novelty profiles and bacterial spectra) could range between $16 – 37 billion [182]. It is nevertheless a proven solution in other sectors for products that are meant to be kept in reserve for use only when needed, for instance in the defence sector (where, in addition to the conservation feature, the other similarity is that the state is the sole buyer).

Still outcome-based but more radical (disruptive) are proposals such as delinking revenue from volume or reimbursements conditioned on meeting conservation targets (an idea developed under the Antibiotic Conservation and Effectiveness (ACE) programme [279]); this would indeed provide a solution to the incentive that drug companies have to promote the volume of their drugs by instead forcing them into abiding by conservation measures. In the same vein as the Antibiotic Corporate Bond, and as disruptively innovative is the proposal put forward by Mossialos et al from the LSE of a Call Options for Antibiotics (COA) [316]. Defined similarly to the eponym financial tool, this call option grants the right to buy antibiotics, at previously fixed prices, during earlier stages of development. This measure has some characteristics of the previously described market guarantee as it can *de facto* represent an advanced market commitment, yet this one is optional and not firm, therefore balancing the risks more evenly between the two parties. This mechanism receives further attention in the discussion around its not-so-far ACB idea, below. The delinkage idea draws on the rationale that a reasonable (or even: reserved) use of antibiotics is essential to limit resistance, hence rendering obsolete the traditional revenue formula based on price and volume. Another principle for return-on-investment will substitute the old economic paradigm, yet this change might not be straightforward. As this has never been tested on this scale in the pharmaceutical world, implementation is likely to be a barrier, and examples or experience can only be drawn from other sectors. Guaranteed markets in the form of lump-sum payments from the governments, as described above, are among the possible solutions. Success factors for the delinkage measure, such as scope, certainty and efficiency, as well as challenging points such as defining value or sharing risk, have been discussed elsewhere [182]. Another idea, integrating the issue of resistance and putting more

[53] This measure was not deemed effective enough and the US introduced another bill, Bioshield II, which puts forth the innovative proposal of wildcard patents (see discussion on wildcard patents in the regulatory part).

emphasis on a ‘service delivery’ rather than just a ‘sale delivery’ ties the lump-sum payment that the drug company receives to service performance. The example also comes from the defence industry, where the government, with the performance-based logistic contracts, is not only buying a piece of equipment, but also the services to this piece [314]. Services in the antibiotic industry could, for instance, take the form of drug-use monitoring or resistance surveillance systems, information of crucial importance in the strategy to combat resistance.

Finally, progressing toward even more innovative, of broader scope models is one more idea encompassing several areas of the antibiotic sphere. The Knowledge Ecology International (KEI) organisation proposed an idea shortlisted by the WHO, the Antibiotics Innovation Funding Mechanism (AIFM) [317]. The AIFM is a proposal that sets taxes or user fees on the use of antibiotic drugs, including human and agricultural uses, and directs the revenue generated to finance a system of grants and innovation inducement prizes. The success of this measure is conditioned on a tight control on end utilisation, with regulations that could take the example of geographic or therapeutic quotas, a decentralized mechanism to manage such quotas, and possible monetization and limited trading of quota amounts.

SECTION 3. INNOVATIVE BUSINESS MODELS: TWO PROPOSALS TO INCENTIVISE ANTIBIOTIC DRUG DISCOVERY

It is admittedly not easy to sort and assess the interest and viability of all these possible measures, and each of them has particular advantages and drawbacks. Advocated in the literature is the combination of several incentives from the IP rights, clinical trials, and financial sphere [281]. However, rather than a siloed view assessing the measures individually, the contextualisation that this thesis has strived to provide with the understanding of the different challenges of antibiotic research and development makes it possible to rough out the type and timing of the required financial incentives. Because of bottlenecks that are mapped from very early stages (scientific issues) to the distant end of the marketed stage (commercial uncertainty), the ensuing incentives are cash flow awards that would be spread across the entire antibiotic’s lifecycle, from prizes to carry out preclinical studies to lump-sum payments or favourable reimbursement measures after approval. Matching this theory are the two novel measures proposed hereinafter in this thesis.

A. NORMATIVE PERSPECTIVES

With any novel scientific departure, it is important to understand the current setting in which it arises – the paradigm it will change. This panoramic view has been analysed in the previous sections, depicting the current state of the art in the multifaceted antibiotic world, from antibiotic development, resistance knowledge, and epidemiology to trade characteristics and economics. This thesis contributes two solutions that are innovative[54] elements to re-engineer the value chain of the antibiotic industry.

When current market forces fail to keep companies interested in the development of novel antibiotic agents, and when these agents have themselves social benefits, there is clearly a case for external intervention (government regulation) and possibly disruptive innovative systems to thoroughly reshape the environment and restructure the dynamics. Creative thinking and innovative policy are at the heart of the needed elements to solve the current lack of incentivisation.

These proposals do not arise following the conclusion that the tools currently proposed and discussed in the literature are inefficient or not doing enough, but really because of my personal opinion, after having explored the field and analysed the possibilities, that a paradigm shift and an all-encompassing solution was needed in order to efficiently and durably fix the problem; that this unique class of drugs calls for a similarly unique solution.

This reflexion started with a thought-provoking piece of work from Chatham House that, to my mind, stood out of the current literature on the topic as it suggested innovative ways to think about the business model of antibiotics [314]. It was further motivated by the understanding that, regardless of their own different merits and drawbacks, the multiple incentives proposed so far do not manage to address a vast majority or all of the unique features of antibiotics, and with non-specific funding mechanisms or granting of regulatory solutions that could as well apply to orphan drugs are we likely to continue to witness a failure in the market. Without disregarding the regulatory and scientific challenges, sustainable solutions would need to integrate the social benefit of antibiotics and fully take account of their market undervaluation, while addressing the revenue generation model and the effects in both livestock management and human health. What exactly would be needed specifications for a useful solution to antibiotic resistance? Recognising the specific inputs at hand and the elements that need to be addressed is possibly the best starting point to formulate positive solutions (the most objective, fact-based response that the theory could dictate) and extrapolate with normative judgements when need be.

[54] These proposals have indeed not been published or cited in any form in the current literature (the PASTEUR idea) or, cited in but a couple of reports, they have not been subject to a thorough and formal analysis (the ACB idea).

This paragraph briefly restates the inputs to the problem. Antibiotics have some unique characteristics that, despite making them unattractive to the industry, cannot be removed; for instance, resistance is always going to develop, and diagnostic will need to play a central part of the infection treatment. Some parts of the business model can be easily improved: clinical trials can be rationalised, as is currently done, or the negative NPV can be compensated by grants or prizes. Fundamentally, as antibiotics are a public trust responsible for positive externalities, they are of social value, which justifies the implementation of public policies and government intervention. Then, who can produce them? The pharmaceutical industry independently has the resources and technology to do so; biotechnologies may have the science but will need partners at some point of the development; and academic laboratories will need subsidies. Secondly, will they? In the current environment, it is unlikely that pharmaceutical companies will engage in the antibiotic production voluntarily: their perception is that they will not be able to make an attractive profit. What are the features of the most appropriate mechanisms that would incentivise the pharmaceutical industry, but doing it in a way as to align the private motivations with the public health interest?

While it is widely recognised that the drug development process is costly, the question of where should the money come from involves a second, more normative one, of who should take the risk for the development. The answer that is provided through the solutions below is that, because of its public good feature, the antibiotic and its production would be best funded if any financing actor was allowed to participate. This includes compulsory engagement from the government side, interested due to the public health aspect of the question, as well as the pharmaceutical industry, through a notion of a particular social responsibility for actors engaged in the health industry, a possibly legally-enacted compulsion for enterprises making a profit on (private) health to contribute to public health. This also includes voluntary engagement, from public investors, for instance on the debt capital market, who will be allowed to express their concern with antibiotic resistance and interest in seeing the armamentum of antibacterial drugs replenished (or a more general interest in public health questions). When funding is collected, any drug company with a strong case for bringing a novel antibiotic to market should be granted the financial opportunity to do so, whether it is a big pharmaceutical or a biotechnology company. The question of what happens after receiving the regulatory approval is perhaps the trickiest one: what should the dynamics of antibiotic marketing look like? Because of the conservation issues that prevent volumes to be pushed upwards, it is not an optimal solution to keep the traditional revenue generation model, based on price times units (another solution would be to reward companies through a higher price, as in the orphan drug model, yet it was chosen not to keep this as a viable solution since there would still be an incentive to grow volumes). Then, there is scope (and a basis) for a re-engineering of the profitability equation. What other approaches could be explored? The one that logically ensues from the inappropriateness of the traditional equation is to delink profits from volume and/or price [**318**].

This approach is the most appropriate from a normative standpoint, yet the practical question of which replacement models to implement as a result concentrates the hardest talk.

Companies can be granted lump sums of money for their successful antibiotic development; this could, for instance, take the form of drug buyout, where the government buys back all the rights and administrates the drug as it sees fit. Stewardship and conservation issues are at the same time addressed with this solution.

On the other hand, different instruments, less blunt and more adaptive, could factor in variables that can have an impact on antibiotic usage. This would take the form of a value-based payment, since the money granted to the sponsor for its drug would reflect the quality of and need for the antibiotic, in the current marketplace, changing if public health conditions evolve (trends in resistance, for example); this is such a structure that the second solution develops in this section.

Regardless of which payment mechanism is chosen, some key conditions remain during the marketed period and lifetime of the antibiotic, such as stewardship and adequacy of the prescription. Still from a fact-based standpoint, also, it should not be the case, at the end of the exclusivity period, that the prescription patterns of the drug surge because of the apparition of generics. This point is briefly addressed by the second solution, however not its main focus.

With the main theoretical concepts necessary to substantially address the drawbacks of the current antibiotic business model in mind, the rest of this section now turns to a couple of practical propositions that have the bold aim of helping fix the antibiotic business model.

B. PROPOSAL 1 : THE ANTIBIOTIC CORPORATE BOND (ACB)

a. Concept

The idea modelled here is that of the Antibiotic Corporate Bond, first introduced by Barclays Investment Bank in a report on innovative business models for antibiotics [314]. The path-disruptive principle of this idea is to raise money from bond (fixed-income) investors, through the issuance of an earmarked bond with special features, the Antibiotic Corporate Bond (ACB). This bond has some of the characteristics of a normal bond: this is a financial instrument that pays interest at regular intervals, and at maturity pays back the initial amount of money that was invested (the principal). This debt instrument, lending money to an entity, is traded on the Debt Capital Market (DCM). This market is the world's biggest source of funding for corporate entities, at the same time largest by its investors' base [319] and deepest by the amount of money invested (more than €180tn worth of assets to invest ($230tn) as of 2012) [320]. It has therefore been critical for providing

capital to corporate structures, pharmaceutical companies included, but the idea behind the ACB is to develop a dedicated financial instrument whose proceeds will be exclusively used for antibiotic development.

Figure 51 explains how the model works. An independent ACB agency issues a basic ("plain vanilla") bond in the debt capital market. The bond will pay coupons at a fixed interest rate before repaying the principal at maturity. Fixed-income investors, such as pension funds, mutual funds, insurance companies or sovereign wealth funds invest in the security. The money thus collected is administrated by an independent agency, which retains a percentage to service the bonds over their lifetime (coupon payments). It then decides how to distribute the remaining money in the form of grants to different biotech or pharmaceutical companies, which need to demonstrate a great probability of successfully developing new antibiotics that meet public health needs.

When a company is successful at getting an antibiotic on the market, a Patent Extension Certificate (PEC) is issued. In a sense, a PEC is a patent derivative, a certificate that allows its owner, a patent holder, to extend the duration of the patent on a protected molecule of its choice. This PEC is transferable, granted to the agency which sells it on the market through an auction process to the highest bidder (pharmaceutical company). The proceeds of the sale are used by the

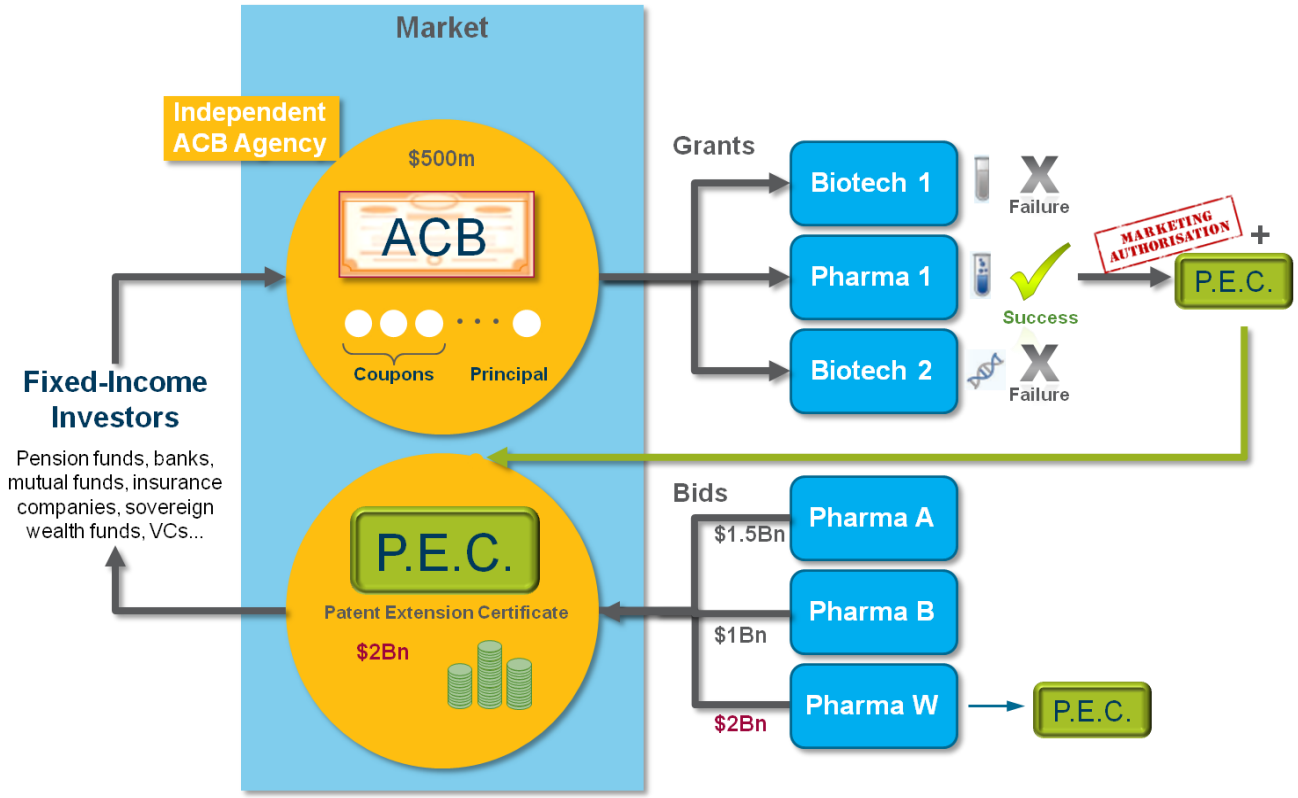


Figure 51. The Antibiotic Corporate Bond: Model. Notes: VC= Venture Capitalists.

agency to reward the successful company and pay back to the investors the initial amount invested (the principal); a percentage is also retained for the agency's administrative functioning, and any extra money is used together with the second cycle of ACB to fund new projects. In the case of unsuccessful projects, the agency is responsible for paying back the investors, initially backed by government funding, and after a few investment cycles using the extra money available. Such a model has no equivalent in real life, nor has it been previously described in the literature. The main purpose of this paper was then to assess the feasibility and financial sustainability of the model, with different cases and cycles, using assumptions and numbers from the literature.

To further explore if this solution could be a sustainable and self-financing one, a modelling task evaluating whether the model makes economic sense has been undergone (unpublished work; details are provided in Appendix 8). Probability-weighted estimates of the amount of money raised, the number of antibiotics successfully developed and the money raised through the auction process of the PEC concluded to the capacity of the model to be beneficial after about five investment cycles, with the ability to fund on average three companies (grants ranging from $110.4m to $306m), with a drug development success rate of about 9.4% and the amount raised from the sale of the PEC of, on average, $4 billion. Although the ACB is unlikely to become self-funding from the first cycle (only 38.5% chance, based on 200 independent simulations), with about 5 cycles needed before profit, its potential for raising money is huge: due to the attractiveness of the PEC to pharmaceutical companies, a number of 5 antibiotics successfully brought to market, which happened after 20 investment cycles here, translates into a profit of $22.5bn for the agency. This ACB model displays the high-risk, high-reward structure typical in drug development, and provided some external support is available at the beginning during the loss-making cycles, it could prove capable of both supporting antibiotic R&D and becoming sustainable.

b. Discussion

Based solely on mathematical modelling, and from a pure economic point of view, it has been demonstrated that the Antibiotic Corporate Bond model is viable. However, the financial logic is just but one part of the model which cannot be considered in isolation. Several other elements, scientific, regulatory or political for instance, are at stake and need to be part of the overall picture on the practical viability of the ACB, with advantages and limitations, in a real-life context.

First, two levels need to be checked regarding the financial aspect: the internal validity of the analytical model, which determines the reliability of our conclusion, and the feasibility in real-life (external validity). The internal validity is discussed in Appendix 8 (Insert 8). The external validity discusses whether such a model would be attractive to investors in addition to the other challenges that may arise in a real-life setting.

Fist, the most impactful assumption in the model is the amount that the sale of the PEC is likely to generate: such a market does not exist *per se* today, and as such, numbers had to be estimated based on our own understanding of the product, the industry and the players, rather than taken from empirically validated data. Barclays believed a $4bn figure was a conservative estimate [314]. There is no pure empirical equivalent against which this estimate can be benchmarked but a special type of transaction witnessed recently in the pharmaceutical sector can provide a good comparable. There have been two historical precedents for the sale of another transferable tool in drug development, namely a Priority Review voucher (PRV). Unlike the Patent Extension certificate, this voucher provides a regulatory advantage, and not an IP rights one. But similarly to the PEC, the PRV was designed based on the same rationale: to incentivise the development of new drugs for neglected diseases (particularly rare paediatric or tropical diseases). Furthermore, it has opened the way for a new market where demand is strong. For the first voucher sold in 2014, Gilead agreed to pay $125m to Knight Therapeutics [321]. And the market value is growing. In May 2015, Retrophin sold its PRV to Sanofi for $245m, almost doubling the precedent price for the voucher [322]. The third and most recent example to date is provided by United Therapeutics, which in August 2015 sold its PRV to AbbVie for $350m, a 48% increase in the PRV market value [323].
It can be argued that the PEC sale will raise to even higher figures, considering the attractiveness of a patent extension: the last years before a patent expiry are the most lucrative to pharmaceutical companies. For instance, 80% of Prozac sales happened during its 5-year patent extension granted by the SPC (Supplementary Patent Certificate) [324]. Sales are usually peaking near patent expiry, and pharmaceutical companies would want to pay a substantial amount to delay the dreaded "patent cliff". Based on worldwide sales at risk for products facing patent expiry ($56bn in 2015, with an expected $19bn sales loss, Figure 52), the $4bn tag price for a patent extension does look quite conservative indeed.

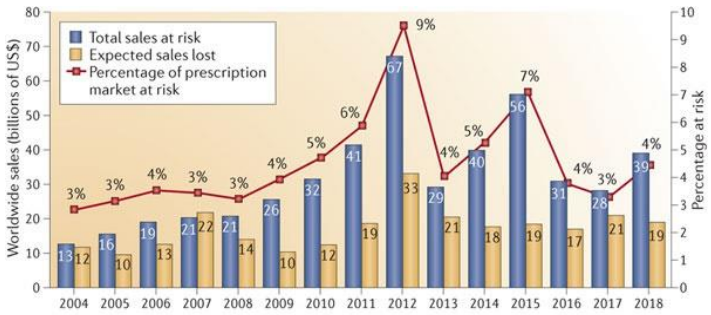


Figure 52. Sales at risk from patent expiry. From: [325].

A second point that the model has highlighted as worth discussing is the attractiveness of, and demand for, a financing device such as the antibiotic corporate bond. The debt capital market is the biggest market for investors with global financial assets of more than €180tn ($230tn) as of 2012

[320]; the reasons for such a popularity compared to the equity market lies in the lower risk attached to bonds, the guarantee of an annual coupon payment at fixed rates and the repayment of the principal at maturity. The ACB has all the characteristics of a plain, standard bond, therefore meeting the fixed-income investors' criteria. Also, it has been proven with initiatives such as the Green Bonds or the Vaccines Bonds that earmarking a financial instrument for specific purposes brings an added value to the investor, in addition to being an effective tool to raise funds. Indeed, since 2008, $8.5bn have been raised in Green Bonds (created to fund environmental-friendly projects) through the World Bank [326]. This could reach an even bigger value if it were made compulsory for companies to dedicate a specific percentage of their R&D to these earmarked programmes.

It is not uncommon to consider financial transactions for health-related programmes. The real-life example closest to the Antibiotic Corporate Bond is perhaps the Vaccine Bonds programme, administrated by GAVI (Global Alliance for Vaccines and Immunisation) and backed by IFFIm (the International Finance Facility for Immunisation). This programme consists of sales of earmarked bonds in the capital market, and the proceeds are made available to GAVI for its global immunisation campaigns. Since 2006, about $4.5bn have been raised [327], translating into tangible clinical results. For instance, the percentage of children receiving the pneumococcal vaccine has increased by 9 points since 2013 in 73 targeted countries [328], and more than 4 million children are expected to benefit from the launch of a polio vaccine in Pakistan, where a 75% reduction in cases compared to 2014 has already been witnessed [329].

Other financial models have been applied to antibiotics specifically, such as the Options Market for Antibiotics (OMA) model [316]. Although this model may succeed in incentivising companies, it does not appeal to the same base of investors, as the purpose of the OMA is to buy the rights to purchase drugs, not a financial instrument, and is intended therefore mainly at governments or NGO, a pool less deep and broad than the fixed-income (private) investors. It makes sense to reach to private investors to fund research, because their interest can be leveraged and aligned to the one of pharmaceutical companies. But their investment decision will take into account the balance between risks and rewards. On the one hand, fixed-income rewards are guaranteed by the coupons and principal; on the other, the issuer of a bond is key to assessing the security's risk. This highlights the importance of the backing of the bond. For instance, Green Bonds are issued by the World Banks, a triple-A rated issuer, and Vaccine Bonds, by IFFIm, rated AA [327], [330]. Investors need to have confidence in the issuer; therefore, in the case of the ACB, it is suggested that the independent ACB be a supranational institution with a similar high credit quality. Alternatively, the World Bank or the European Investment Bank could also qualify to take on the issuance of ACBs. Unlike Green or Vaccine Bonds, ACB's proceeds are to be used by corporate entities, as their name suggests; accountability has to be rigorously monitored to ensure the appropriate use of the funds.

Systems will have to be put in place, and whether or not to make them legally binding is up for debate. As an aside, Green Bonds' uses of proceeds as not legally binding, but no misallocation has been reported so far.

To conclude on the financial part of the discussion, it is worth noting that other financial instruments, such as drug royalties securitisation, have been suggested and described as potential solutions to incentivise R&D in various therapeutic areas; although most have not reached the implementation stage yet, they at least open the dialogue on innovative solutions for the funding gap [331], [332], [333].

There are therefore some precedents to provide confidence in the real-life feasibility of the financial aspect of the ACB. But what about the regulatory aspects? This is the third aspect of the discussion. The ACB model is based on the controversial idea of wildcard patents. Their advantages and drawbacks have been analysed in the regulatory section (Section 2 B). Among their main consequence is the fact that such regulatory devices create externalities: by linking antibiotic development to rights on other, non-antibiotic drugs, the cost burden is imposed on society (more specifically, on patients taking the non-antibiotic drug – or their health systems). Illustratively, the costs to society of this measure could be as high as $40bn if ten wildcard patents are granted [283]. But if one only cares about the financial impact of the measure, a compensation solution might easily be found. According to the mathematical model, ten new PECs will have been granted by the end of the 28th investment cycle. By that time, the money accumulated by the agency would have reached $42bn. Part of this pool could, therefore, be used to compensate the additional costs incurred to public health budgets. This could take various forms, for example through donations to governments' social security institutions. If the questions of fairness and equity could be addressed at the same time by such a financial correction, then the measure would appear less unattractive.

Because there are still several inefficiencies with the patent extension certificate, the antibiotic corporate bond idea could be improved by a change in the repayment mechanism. One solution, allowing the idea to keep its core structure as described, would be to replace the transferable patent by a regulatory voucher, for instance (a Priority Review or Fast-Track voucher). It is likely that the money raised through the sale of these different incentives would be lower, but the model has not been run to assess the viability of this alternative scenario. Additionally, the repayment mechanism could also take a structure completely different from the current one based on the sale of regulatory incentives. For instance, a more radical revamp would be to remove the reward associated with the antibiotic approval and just sell earmarked antibiotic bonds (this is the business model of Vaccine Bonds), which would be guaranteed by the company's entire balance sheet, with however the risk of not generating enough attention, and not enough capital, from companies and investors. Further

work and creative ideas are welcome in this area. The second proposal, at the end of this section, draws on the antibiotic corporate bond idea with a different repayment mechanism.

c. Policy implications

Lastly, the political feasibility of this model is perhaps the most uncertain and questionable one. Why would governments agree to support a measure that, among its drawbacks, involves a financially risky commitment from them in the first cycles or delays generic entry?

The ACB might be technically financially viable and feasible in practice if it can follow the successful examples of its Vaccines or Green predecessors, but it will require much public willingness and stewardship. Government involvement is required when market fails, as it is the case here (see Section 2), but even more needed in the case of disruptive innovation pathways, to shape a new market that private investors can move into [300], [334]. It is government's role to not only lead the way and tailor the new system but also take part in its effective governance. For instance, the IFFIm could not be such a crucial partner to GAVI in the vaccines bond segment if it were not backed by 12 governments, including the UK, Canada, and France [327].

Governments have several roles to play in the ACB system. First, they would need to set up or ensure the election of an appropriate independent ACB agency to issue and service the bonds, allocate the grants and auction the PEC. Supranational agencies, if not included in the management of the ACB agency already, will also need to oversee the functioning.
Secondly, there is an extensive role for public bodies in the implementation part, notably when it comes to the distribution of the grants. What projects should be chosen? A framework for selection, including the definition of needed antibiotics (and the criteria for "need": is it a public health threat, based on the burden of disease or economic impact?), business case assessment, evaluation of the company's success probability, scientific, clinical and technical capabilities, etc. In the model, only companies that had passed the preclinical stage were selected; the attrition rate is too high in the preclinical trials to mitigate the risk on the capital markets.
Furthermore, the concept of the PEC (or wildcard patents) need not be seen as completely negative if governments can define its terms and conditions. For instance, the duration of the extension will require careful thinking: what is the optimal duration to balance the needed incentive with the consequent social costs? The scope of the PEC can also be framed, e.g. it could only apply to a selection of chronic diseases. Also, where would the certificate be valid? Is it for the bidding company, the ACB agency or the governments to choose?
Nevertheless, it is important to note the flexibility of the system: if PECs are deemed too controversial to be supported, other solutions might be implemented, as addressed at the end of the above Discussion.

Public bodies are also responsible for addressing the question of equity: what could be done to limit the negative costs resulting from the generic delay? Should the profits controlled by the ACB agency be used, and if so, how?
And even more importantly: what is the future of the newly developed antibiotic? It has been bought out by the lump sum rewarding the successful company, but whose responsibility does it fall under? What stewardship systems will take over from there?

There are lots of policy implications to consider thoroughly. The ACB model may as well raise more questions than it answers, but a key one remains: will it stimulate action?

C. PROPOSAL 2 : THE PASTEUR INITIATIVE

a. Concept

Advocating the need for a more global solution encompassing the whole of the antibiotic business model components and not just its financing part, rooted in the idea that profit from antibiotics should be unrelated to sales, that reward should be correlated to appropriate use in order to responsibilise industrials as well, and that the recognition of the public health value of antibiotics should be as important as the diversification of financing sources is the Pan-sector Antibiotics Sustainable Trade whose Economics Utilise Resistance, or PASTEUR idea.

Designed to bring together most of the theoretical requirements necessary to improve the business model of antibiotics, this concept is a sector-wide proposal that addresses challenges across the entire value chain of antibiotics, embracing the view that the bunch of innovative solutions to fix the antibiotic business model should be integrated in a novel and dedicated framework, in order to work efficiently. Two innovative features are included: the antibiotic tax and the resistance-based reimbursement.

At the beginning of the chain with the financing issue is the PASTEUR idea building on the ACB one (Figure 53). Recognising the value of implicating a broad base of investors, not least to sensibilise a wider range of stakeholders to the issue of resistance, the framework includes access to the debt capital market and the issuance of earmarked bonds for antibiotic development. Most of the features from the ACB are kept, including the overarching governance of an international organisation, tasked with the gathering, administration, and allocation of the funding (grants to various drug companies with a strong case for successfully bringing a novel antibiotic to market). It departs from the ACB model in the repayment mechanism: the earmarked bond would be government-backed, a guarantee for the repayment and a solution to involve state institutions and

allow them to play their role in the management of the public goods that antibiotics represent, and financed by the pharmaceutical industry.

This mechanism, first innovative feature, takes the form of an "antibiotic tax", representing for instance 1-5% of the industry's profits, sensibilising them to the public health role that they have, in addition to the private health role that they might address more readily. The financing help to antibiotic drug manufacturers takes the form of grants to companies with a strong investment case (high probability of bringing a novel and needed antibiotic to market). Then, once approved, the marketed antibiotic would be valued through an innovative economic system, and the sponsor paid accordingly. The second innovative feature that the PASTEUR idea puts forward states that the reimbursement is based on the value that the novel antibiotic brings to society in the light of the current bacterial sensibility and resistance levels. Value is here defined as a function of the interest that the drug represent, its efficiency evaluated through the current drug resistance levels (and to which value is inversely proportional); it is correlated with the gap that the drug fills

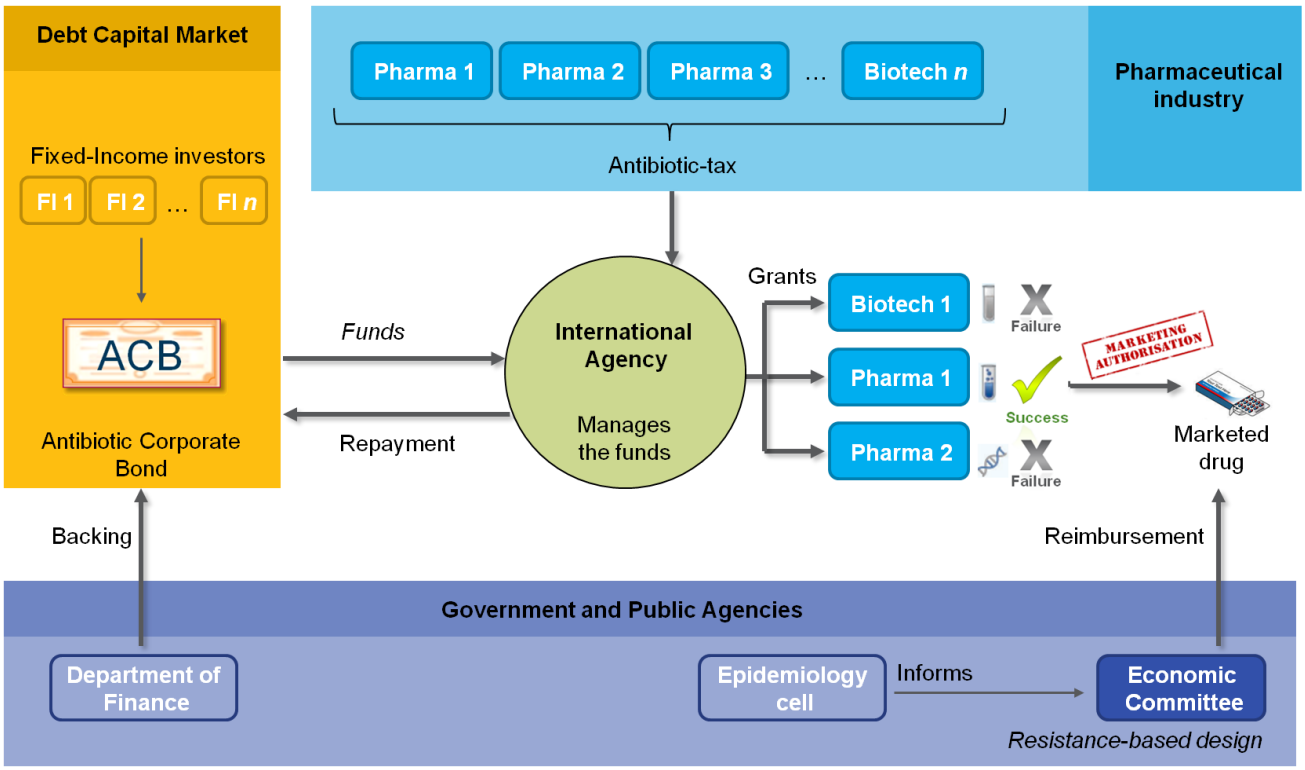


Figure 53. The PASTEUR initiative.

between the current, available therapeutic arsenal and the needed antibacterial agents. The drug is marketed in the first years according to stewardship practices; then, if resistance to the drug is high, the reimbursement that the government gives to the sponsor is low, because the drug is not of much value since epidemiology predicts that bacteria are currently highly resistant to the drug. If

resistance is low, the drug is to be effective in most of the cases, translating into a high value and a higher reimbursement. The innovation of this payment mechanism resides in the variable included in the reimbursement formula, not a function of consumption and volumes (with price) but a function of resistance.

b. Discussion

The heterogeneity of antibiotic externalities makes the problem of antibiotic resistance difficult to administrate. The theoretical solution, as described throughout Section 2 and the beginning of Section 3, would require a delinking strategy, where the return from the antibiotic drug is not to be conditioned upon sales volume, to avoid the incentive for overuse. Additionally, the intelligence gathered from economic theory strongly suggests that payments (taxes, prices, rents) distinguish between uses (low-value, high-value uses) and to correspond to the particular externality.

However, arbitrage within community pharmacies or hospital units is quite hard to implement. Differential payment based on the appropriateness of the antibiotic prescription would require an extensive amount of data on each clinical situation, not to mention the fact that with empirical treatments there is not even reliable information to distinguish between low- and high-value usages. Such a differentiation at the micro-level is, therefore, inconceivable. But this setback does not mean that the tier system cannot be implemented at all.

The PASTEUR initiative addresses this issue with a reimbursement system that does not depend on consumption through the proxy of volumes, a poor design for drugs subject to resistance, but on real-life effectiveness through the proxy of resistance levels to the drug. Such a design rearranges the profitability equation from the traditional price times volume to a function of resistance, dynamic and linear. In practice, the information on resistance trends will come from an epidemiological cell on a yearly basis. Building on this input will the economic committee for drug reimbursement propose a figure to the sponsor. On the years that resistance to the drug is low, such as in the early years of marketing or thanks to a suitable stewardship campaign, the company will receive a substantial payment. If the resistance is high, after several years or because of an aggressive promotion to boost volumes, the payment is low. In the first couple of years where the drug has just launched (resistance close to zero) and as a baseline for the subsequent payments, the drug could, for instance, be valued relative to the overall resistance levels among the bacterial strain it targets: the more resistant the bacteria are, the more valuable the novel drug will be.

This reimbursement mechanism will provide different incentives on each party. For the government, despite the opposite reasoning, it still stems from the broader picture of the resistance problem that tackling it is the more sensible action to take, with the reduced costs from the associated extra interventions and healthcare consumption offsetting the relatively higher

reimbursements for low-resistance levels. For the sponsor, there will be an incentive to act in order to curb resistance, as the money reimbursed is increased in the case of low resistance levels. This novel idea would, eventually, entice sponsors to market not only their antibacterial drug on its own, but with a framework of actions against anticipated resistance, since they are incentivised to delay the apparition of resistance as much as possible.

Another point addressed by this initiative is the redistribution capabilities conferred not only through the grants but also via the antibiotic tax system. The justification for two such mechanisms is that distributional dynamics and social welfare would be enhanced if funds were pooled globally: indeed, there is a discrepancy between the financing and the antibiotic drug discovery capabilities among the various actors in the pharmaceutical industry, where companies with an important liquidity cushion do not necessarily have the technology or innovative features to uncover a novel molecular entity. The funds, collected on a voluntary (bonds) or compulsory basis (antibiotic tax), are redistributed by a competent body, the international agency, which has access to the business cases of the antibiotic companies and whose experts can objectively discriminate between the high – and low – success probability to develop a novel antibiotic.

Turning to the second innovative feature of this model, the antibiotic tax has been chosen for several reasons and has implications worth discussing. First, it is one of the economists' most efficient tool when it comes to dealing with goods with externalities. A telling example is the "eco-tax" on motor fuels, which impact the prices that car users are facing and thus have important environmental impacts. Additionally, major global airlines are charged for their carbon emissions since 2012 under the EU Emissions Trading System [335]. These taxes are designed to reflect the size of the negative environmental externality associated with fuels [336]. Another example comes from the tobacco industry, where another negative externality, passive smoking (second-hand smoke), justifies some of the taxes imposed on the cigarette packs[55]. Although the clinical market for antibiotics is much more complex, with a mix of externalities and a public health need for such drugs, it is nonetheless theoretically justified, due to the negative externality arising with usage of antibiotics, and in line with what is currently done in similar industries, to create a tax on the antibiotic industry.

Also, a more normative justification follows on from a concept of social responsibility for industries engaged in the health field. Medical suppliers respond by focusing their efforts on products that will appeal to a broad market of paying customers (the "private health" side). But they also have a broader, and often overlooked, social responsibility, which relates to public health actions. For instance, federal tax law in the US requires that hospitals perform a charitable function in their communities in order to retain their tax exemption [337]. The notion of community benefit, or social

[55] Further lessons can be drawn from tobacco control where a combination of taxes, education and legal restrictions has reduced utilisation.

responsibility, may be instilled in law or it may not, but the core principle that a few public health actions may rest upon healthcare and health-technologies industries in the broadest sense is not inconceivable. No modelling work was undertaken for this proposal, but the question of whether the notion of an antibiotic tax makes financial sense or not can be indirectly addressed with some estimates provided by the Review on Antimicrobial Resistance. According to their calculation, the annual pharmaceutical revenue for the ten largest pharmaceutical companies amounts to $429.4 billion, while the cumulative profit figure is $89.8 billion. As an illustration (and keeping in mind that this figure only represents the first ten pharmaceutical companies), a 1% tax on profit would raise almost $1 billion. Any tax requires a careful design, and while it is recommended that the tax be imposed on every pharmaceutical, healthcare or health technology company, exemptions could be made for companies that are already engaged in this therapeutic area or dedicate a percentage of their R&D efforts to antibacterial drug (or diagnosis) discovery.

The amount that such a tax would be able to raise calls into question the necessity of other financing tools, such as the antibiotic corporate bond that is included in the initiative. Several alternative propositions have been assessed, apart from the standalone ACB proposal described in Section 3 B, such as the ACB model with a repayment mechanism disconnected from the final authorised antibiotic, the ACB model with the antibiotic tax as a repayment mechanism, a simple earmarked bond with no other guarantee and the removal of the ACB feature altogether. The question then becomes a normative one, and it can become quite subjective to assess whether this bond element is needed. In this thesis, it is advocated that the ACB remains in the framework, since, regardless of any question on its financing efficiency, it has at least the benefit of engaging other stakeholders around the question of antibiotic development and raises awareness in a broader community. Further research around this feature such as a modelling of the costs involved could shed some more light on the question and tip the scales in favour of one side or the other.

Overall, the proposal fulfills several conditions of the appropriateness evaluation criteria (as put forward by Sertkaya et al [281]). With the availability of funding, the diversification of the sources, and the sensible reimbursement mechanism during the marketed period, it improves the antibiotic R&D net present value (NPV), which was an essential criterium preventing companies from investing in the first place. Secondly, it aligns the private incentive with the public incentive, limiting market distortions by basing the reimbursement level on the resistance levels, not on the consumption (volumes) level. This feature also encourages antibiotic conservation and appropriate usage. Thirdly, it encourages the involvement of all the players of the antibiotic industry, from small enterprises to large-cap firms (through the antibiotic tax and the possibility to be granted funds for R&D). With the initial reimbursement level depending on the overall resistance profile of the bacteria targeted is the industry incentivised to develop new drugs against the most resistant

bacteria, even anticipating the forthcoming resistance development, therefore stimulating valuable innovation and competition as well as targeting specific high-priority antibiotic needs.

This proposal, however, has several limitations. First, it is not exactly clear whether it would be sustainable, and quantitative analyses are much needed when it comes to further research.
Secondly, it does not address other aspects of the multi-faceted problem, such as prescription patterns and conservation measures, or the use in the agricultural sector. Regulatory measures such as changes to clinical trials or to the patent system have not been included, for one thing because it is now believed in the industry that the regulation on clinical trials is no longer a main issue (see Section 2 B), and also because some key reasons to advocate for a change in the patent system (for instance, the incentive of pushing sales during the few remaining years of marketing exclusivity) are no longer relevant in this framework, since revenue and profitability are delinked from volumes.
Thirdly, it can be argued that the proposal would not be easy to implement, notably with the need for a dedicated, capable organisation with oversight and expertise capabilities as well as competent governmental bodies to ensure a fair reimbursement.
Additionally, the result on patient access of the various incentives that the model creates is not completely clear and needs further reflection. The first conclusion that comes to mind is that since profitability is delinked to volumes, there is no incentive to restrict or promote usage, but the reimbursement mechanism may not be prevented from creating less visible perverse incentives.

c. Policy implications

Funding will continue to flow from public, national budgets, yet more coordination is required to engage other financial and investment actors. In addition to allowing the debt capital market, through the backing of the antibiotic corporate bonds, to participate in the funding of novel therapies, the government would support this novel structure in the antibiotic business model by addressing its current national budget and other dedicated funding sources for antibiotic development to the international, overarching agency, therefore delegating the screening, selection and allocation activities, removing the need to fund such resources as well as handing down the risk involved in the process.

Because the innovative reimbursement mechanism is based on national resistance trends, a strong political will to commit resources to the gathering and analysis of good-quality epidemiologic data is essential. This effort could, for example, be financed by the funds redirected from the grant-allocating capabilities that are no longer needed (previous paragraph). Where such data is unavailable, international data or trends from neighbouring countries could be used, highlighting that governments could further enhance the framework by a collaborative work. When it comes to the political implementation feasibility of the reimbursement mechanism, there is the additional

argument on the government's side that since most of the companies marketing an antibiotic would be likely to have been helped by grants during the development part, their risk-taking part is reduced, which justifies a reduced reward as a consequence (whether the reward would indeed be reduced in practice is not even certain and would require a quantitative modelling).

The set-up of a tax on profits that the healthcare industries generate could prove to be a double-edged sword difficult to implement and associated with political risk. Whereas the public opinion would possibly be favourable to the measure, it is not unlikely that the powerful lobbies from the pharmaceutical industry, especially in the US, use their influential muscle to bring the initiative down. Highlighting the particular force of the measure, its justification rooted in the sense of public health duty of these companies, could be of help to the government, even if it does not solve the practical implementation issues.

To sum up, the interconnection of the different pieces of the antibiotic business model puzzle, highlighted throughout this Part, provides a rationale for a different allocation of society's protective and curative resources in order to bring to market the antibiotics that are needed to efficiently fight bacterial infections and curb the public health problem of bacterial resistance. Redistributive mechanisms, involvement of other financing actors such as the debt capital market or a re-engineering of the profitability equation, delinked from volumes but correlated to resistance levels, are some of the most promising leads to improve the economic environment for antibiotics and provide greater total benefit in the fight against infectious disease.

There is no magic-bullet-type incentive or framework that would fix at once and with the least frictions the regulatory, economic and scientific challenges that antibiotics developers face. Still, judicious regulation can help ensure that antibiotics are not prevented from reaching the market when they display an appropriate and reasonable security, efficacy and quality profile, that they are reserved for circumstances where they are needed, and that the most appropriate treatment is provided. The evolution of science and its techniques is a source of objective enthusiasm for future drug discovery. Intelligent economics align the private and the public incentive. The optimal strategy to ensure antibiotic development is multi-pronged, and each aspect could individually be controversial. Whatever framework is pursued, it must synchronise with current and future institutional capacity in order for it to work effectively.

CONCLUSION

ENGAGING THE WHOLE OF THE HEALTHCARE CHAIN : A WAY FORWARD FOR THIS PUBLIC HEALTH PROBLEM ?

The resistance problem through the lens of the market

Although very brief on the bacterial lifespan, more substantial and preluding to modern therapy on the human contemporary history scale, the "antibiotic era" has nonetheless gone through many ups and downs, forcing us to further our understanding of the bacterial world. Life according to these prokaryotes is all about escaping the chemical weapons that antibiotics represent; according to us, it is about outwitting our unicellular opponent, and this ultimate goal encompasses a broad array of tasks, starting from gathering as much intelligence as possible (on how our drugs are doing, on how bacteria are evolving) to nurturing the market and industrial system for the introduction of new drugs.

The development of resistance is closely entangled with the dynamics of the market for antibiotic drugs. Market creates resistance, and resistance creates markets. Additionally, the development of resistance is an evolutionary inevitability: we cannot stop it from happening. What we can do though, and what is important that we do, is acting to slow its spread and contain its impact, and to bridge the gap between science and policy.

I personally join with K. Outterson in his recommandations to the House Energy and Commerce Committee (September 2014): we have to be bold, think beyond the pill and offer substantial incentives. This thesis has provided a perspective and possible solutions on what society and the pharmaceutical industry can do against this concerning public health problem; the one that it particularly values and stands for is the delinkage strategy supplemented by a value-based (resistance-based) payment to the manufacturing company. It also advocates that we need the pharmaceutical industry, vector of innovation and commercial muscle, to engage again in antibiotic R&D. The investment rationale should not revolve around the question of whether an antibiotic can be a blockbuster – Augmentin, the biggest selling antibiotic to date, had yearly peak sales of just under $2 billion –, yet it should lend a more attentive ear to the reasoned argument of the public health responsibility. If the healthcare and health-tech industries are admired for being industrial jewels with groundbreaking science, they are nevertheless evolving in the very particular field of human health, particular with regards to its unmatched level of regulation and its record-high rewards, particular also because of its third component that is often overlooked: their dealing with

human health, improving and shaping our condition like no other industry. These companies must hence be reminded that human health encompasses two dimensions, private health and public health; and while the most profitable areas lie within non-communicable diseases, infectious diseases and their public health impact are not to be brushed aside because it is most convenient for their business do so. The portfolio of pharmaceutical companies, when it comes to them, should contain (lucrative) medicine, together with societal drugs. Whether we need to go to the point of punishment for these companies who do not fulfil their public health duty is open to debate [338].

Putting solutions into perspective: engaging the whole of the healthcare chain

Investigating mechanisms of resistance is essential to understand how the phenomeon arises, spreads and disseminates, and consequently equip decision-makers with tools efficient in curtailing it. It has been the argument of this thesis that the investigation and development of new drugs in the appropriate environment can contribute to the answer to resistance. Yet, because of the high cost and lengthy timelines, this answer is a necessary but not sufficient solution. To put things into perspective, comprehensive and immediate actions are needed.

Of equal importance in providing solutions to the antibiotic resistance challenge are the stewardship efforts for antibiotics. The judicious use of these valuable, public health drugs is the responsibility not only of hospitals and clinicians, but also of the community, agriculture and any other concerned sector that has a profound impact in the development and dissemination of antibiotic-resistant organisms. Appropriate and rational use of antibiotics has to be embedded in the course of therapy for infectious diseases. It may be the case that, to successfully implement and oversee these measures, a broader framework be developed. Justified by the fact that antibiotics are different from all other medicines in that the effects of their use extend far beyond individual patients and that, apart from vaccines, there are no other drugs displaying the unique societal effects of public goods, this framework could, for instance, take the form of a special regulatory category. This earmarking would go hand in hand with specific measures such as controlled prescription pathways, financial rewards and penalties, and accountability from prescribers. Designing such a framework and ensuring that the right incentives are introduced at every level of the healthcare chain calls for further research.

Hence, in addition to innovation, the education and control that stewardship represents is the other face of the dual solution to be implemented, as conservation without innovation will in all likelihood constrict the market for antibiotics, not least by deterring investment. Society has therefore the duty to foster the development of new antibacterial therapies, whether they be traditional small molecules, bacteriophages, accompanying diagnostics, to cite but a few. Nevertheless, adding a

third part to the system of solutions are academics going further, with access argued to be as necessary as conservation and innovation. This area has not been developed in detail here either, and further work on this topic would be most welcome.

From whichever way we look at it, whether standing on a tripod or recognising a different number of solutions, antibiotic resistance is a challenge that requires international and inter-sector collaboration with several aspects to be tackled simultaneously.

Engaging the public

For one thing, the resistance problem represents a complex system, where the difficulty in discovering new molecules is not an isolated scientific issue that could be fixed with technological advances; because it has numerous additional inputs, it requires concerted efforts from clinicians, policy makers, regulators, the agricultural sector, and, ultimately, the public. The WHO has recognised this need with one of the five strategic objectives of its Global Action Plan on Antimicrobial Resistance revolving around improving awareness and understanding of the issue to promote behavioural changes. This objective could, and should, be applied to the general public, and it would be a prerequisite before empowering society to stand for their much-needed antiinfective drugs.

Highlighted in the Introduction was the complacent use of the drugs and the poor awareness of the resistance problem that the public has. Advocated in this Conclusion is the fact that society must seize control of its antibiotics. Although lessons (and incentives) for the public have been very much left out from this thesis, for the non-negligeable role that it can play and the importance of changing attitudes when addressing the issue of resistance is the public a group that is paramount to target. Determining how and from whom will changes be taught remains the core of the problem; for one thing, didacticism, proximity, real-life applications are key. Maybe the best advocates will come from the least expected ones. On June 01, 2016, Pizza Hut announced its plan to make the chicken used for their pizza topping free of human antibiotics [339]. What if advertising campaigns and actions to limit the spread of resistant bacteria were advanced by KFC or McDonald's? It remains to be seen how they could engage the public, but by the breadth of their reach and the quasi-universality of their consumer pool could these brands really make an impact; these moves have the merit to start the ball rolling and will undoubtedly have positive consequences for the drugs that are the cornerstone of our modern therapeutic arsenal.

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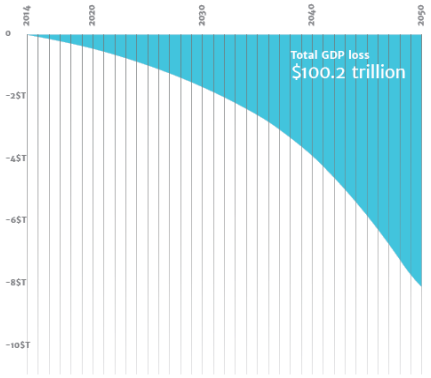
APPENDIX 1

Epidemiological threats of some resistant bacteria

Bacterium	Resistance to	Threat
Clostridium difficile	Fluoroquinolones	Urgent
CRE	3rd generation cephalosporins, carbapenems	Urgent
Neisseria gonorrhoeae	3rd generation cephalosporins	Urgent
Acinetobacter	β-lactams incl. carbapenems	Serious
Campylobacter	Ciprofloxacin, azithromycin	Serious
ESBLs	Penicillin, cephalosporins	Serious
VRE	Vancomycin	Serious
Pseudomonas aeruginosa	Resistance to nearly all or all antibiotics incl. aminoglycosides, cephalosporins, fluoroquinolones, carbapenems	Serious
Non-Typhoidal Salmonella	Fluoroquinolones, ceftriaxone	Serious
Salmonella typhi	Ceftriaxone, azithromycin, ciprofloxacin	Serious
Shigella	Fluoroquinolones	Serious
MRSA	Methicillin and related (nafcillin, oxacillin)	Serious
Streptococcus pneumoniae	Penicillin	Serious
VRSA	Vancomycin	Concerning
Group A *Streptococcus*	Clindamycin, macrolides, tetracyclines	Concerning
Group B *Streptococcus*	Clindamycin, erythromycin, vancomycin	Concerning

Table 1 . Bacteria resistance profiles and threat levels. From:[1], [2].

A.



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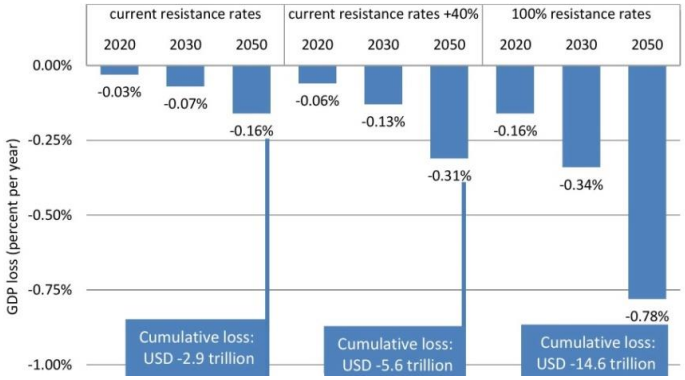

Figure 1. A: Antimicrobial resistance impact on World GDP. Data in trillions of USD. Retrieved from the Review on Antimicrobial Resistance [3]. B: Antimicrobial resistance impact on OECD countries: % GDP loss per year and cumulative losses. From [4].

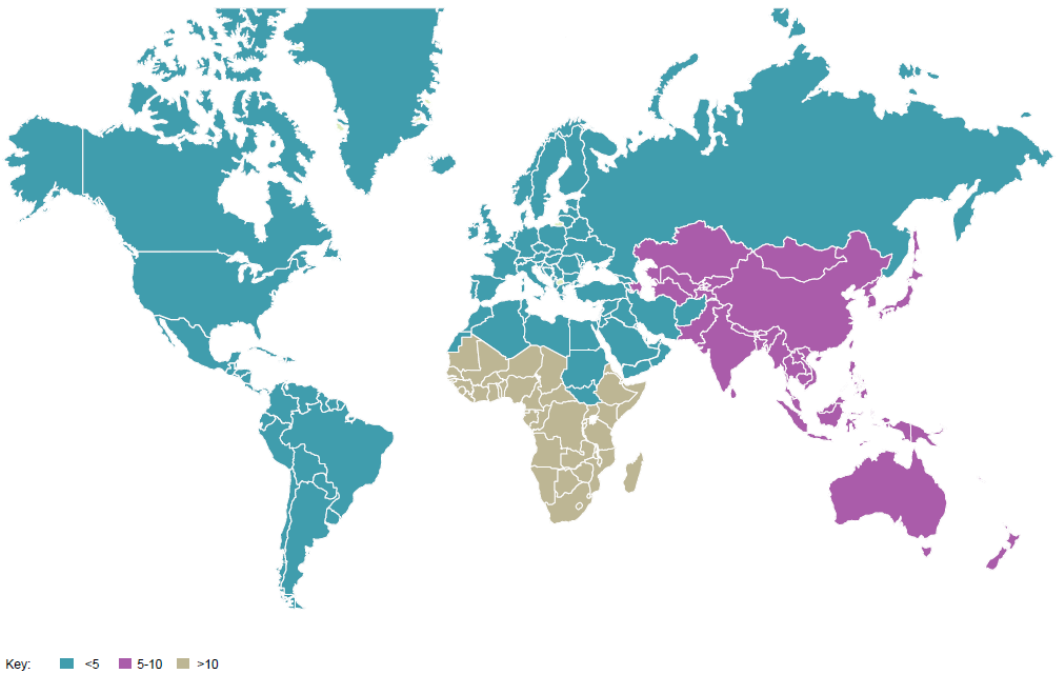

Figure 2. Maximum reduction of GDP in 2050 due to antibiotic resistance. The resistance rates were forecasted as follows: a doubling in current infection rates for S. aureus, E. coli and K. pneumoniae, as well as HIV and tuberculosis, with a 100% resistance rate for all countries. From [5].

APPENDIX 2.

The Golden Age of Bacteriology

Year	Disease	Organism	Discoverer
1877	Anthrax	*Bacillus anthracis*	Koch, R.
1878	Suppuration	*Staphylococcus*	Koch, R.
1879	Gonorrhea	*Neisseria gonorrhoeae*	Neisser, A.
1880	Typhoid fever	*Salmonella typhi*	Eberth, C.
1881	Suppuration	*Streptococcus*	Ogston, A.
1882	Tuberculosis	*Mycobacterium tuberculosis*	Koch, R.
1883	Cholera	*Vibrio cholerae*	Koch, R.
1883	Diphteria	*Corynebacterium diphteriae*	Klebs, T.; Loeffler, F.
1884	Tetanus	*Clostridium tetani*	Nicholaier, A.
1885	Diarrhea	*Escherichia coli*	Escherich, T.
1886	Pneumonia	*Streptococcus pneumoniae*	Fraenkel, A.
1887	Meningitis	*Neisseria meningitidis*	Weischselbaum, A.
1888	Food poisoning	*Salmonella enteritidis*	Gaertner, A.
1892	Gas gangrene	*Clostridium perfringens*	Welch, W.
1894	Plague	*Yersinia pestis*	Kitasato, S.; Yersin, A.
1896	Botulism	*Clostridium botulinum*	van Ermengem, E.
1898	Dysentery	*Shigella dysenteriae*	Shiga, K.
1900	Paratyphoid	*Salmonella paratyphi*	Schottmüller, H.
1903	Syphilis	*Treponema pallidum*	Schaudinn, F.; Hoffmann, E.
1906	Whooping cough	*Bordetella pertussis*	Bordet, J.; Gengou, O.

Table 2. The "golden age" of bacteriology with the discoveries of the main bacterial pathogen; from Brock [6].

APPENDIX 3.

Families of antibiotics

Class	Mechanism of Action	Bacteria Covered
Penicillins (natural) (penicillin G, pen VK)	Bactericidal Inhibit cell wall synthesis (inhibition of cross-linking of peptidoglycan by inactivating transpeptidases, PBPs)	**Gram(+)**, except staph Some **anaerobes** *N. meningitidis*
Penicillinase-resistant penicillins (methicillin, nafcillin, dicloxacillin)	*Id.*	**Gram(+)**, used mostly for staph, but not MRSA
Aminopenicillins (ampicillin, amoxicillin)	*Id.*	**Gram(+)**, but not MRSA Some **Gram(-)**, not *Pseudomonas* Some **anaerobes**
Aminopenicillins with beta-lactamase inhibitor (ticarcillin/clavulanate, piperacillin/tazobactam)		Better staph coverage Better **Gram(-)** & **anaerobic** coverage
Antipseudomonal penicillins (ticarcillin, mezlocillin, pipracillin)	*Id.*	**Gram(+)**, but not staph Some **Gram(-)** Some **anaerobes**
Antipseudomonal penicillins with beta-lactamase inhibitor (ticarcillin/clavulanate, piperacillin/tazobactam)		Better staph coverage Better **Gram(-)** & **anaerobic** coverage
Cephalosporins 1st-generation (cephalexin, cefazolin, cephradine)	*Id.*	**Gram(+)**, but not MRSA Some **Gram(-)** Some **anaerobes**
2nd-generation (cefuroxime, cefoxitin, cefaclor, cefprozil)		**Gram(+)**, but not MRSA **Gram(-)**, not *Pseudomonas* **Anaerobes**
3rd-generation (ceftriaxone, cefotaxime, ceftazidime, cefixime)		**Gram(+)**, but not MRSA **Gram(-)**, weak against *Pseudomonas* Some **anaerobes**
4th-generation (cefepime)		**Gram(+)**, not MRSA or enterococcus **Gram(-)**

Carbapenems (imipenem, meropenem)	*Id.*	**Gram(+)**, not MRSA **Gram(-)** **Anaerobes**
Monobactam (aztreonam)	*Id.*	**Gram(-)**
Glycopeptides (vancomycin, teicoplanin)	Bactericidal Inhibit cell wall synthesis (binding to terminal D-Ala–D-Ala & prevent incorporation into growing peptidoglycan)	**Gram(+)** Some **anaerobes**
Fosfomycin	Bactericidal Inhibit cell wall synthesis (inhibition of cytoplasmic precursors)	**Gram(+)** **Gram(-)** aerobic
Polymixins (polymixin B, colistin=polymixin E)	Bactericidal Inhibit cell membrane function (increase permeability by a detergent-like action)	**Gram(-)** primarily
Fluoroquinolones (ciprofloxacin, ofloxacin, norfloxacin)	Bactericidal Inhibit DNA gyrase & topoisomerase	Some **Gram(+)**, Staph but not MRSA **Gram(-)** Some atypicals
Extended-spectrum fluoroquinolones (levofloxacin, gatifloxacin, moxifloxacin)		**Gram(+)** **Gram(-)** **Atypicals** Some **anaerobic** coverage
Rifamycins (rifampin, rifabutin)	Bactericidal Inhibit mRNA synthesis	*M. tuberculosis*
Macrolides (erythromycin, azithromycin, clarithromycin)	Bacteriostatic Inhibit protein synthesis (bind to 50S ribosomal subunit)	**Gram(+)**, but not MRSA Some **Gram(-)** **Atypicals** Some **anaerobes**
Lincosamides (clindamycin)	*Id.*	**Gram(+)**, not MRSA **Anaerobes** ***Mycoplasma***
Oxazolidinones (linezolid)	*Id.*	**Gram(+)**
Chloramphenicol	*Id.*	**Gram(+)** **Gram(-)** **Anaerobes** ***Rickettsia***

Streptogramins (virginiamycin)	*Id.*	**Gram(+)** mainly
Aminoglycosides (gentamicin, streptomycin, tobramycin, amikacin)	Bactericidal Inhibit protein synthesis (bind to 30S ribosomal subunit)	Staph (combine with beta-lactams) **Gram(-)**
Tetracyclines (tetracycline, doxycycline, minocycline)	*Id.*	Some **Gram(+)** Some **Gram(-)** **Atypicals** Some **anaerobes**
Sulfonamides (sulfadiazine, sulfamethoxazole/trimethoprim*)	Bacteriostatic Folate antagonist/inhibits folate synthesis	Some **Gram(+)** Some **Gram(-)** Some **protozoans**
Metronidazole	Bactericidal Toxic to cells by interfering with electron transport/producing free radicals	**Anaerobes** Some protozoans and parasites
Nitrofurantoin	Bacteriostatic or bactericidal depending on concentration Bind to macromolecules	**Gram(+)** **Gram(-)** Only in the lower urinary tract

*Table 3. Classification of antibiotics, with examples of molecules, according to their mechanism of action and spectrum of activity. *: while trimethoprim belongs to a different chemical family (diaminopyrimidines), it is used in combination with sulfamethoxazole, hence cited there. Adapted from [7] and [8].*

Pharmacology of antibiotics

Action on cell wall synthesis

The peptidoglycan layer is assembled using basic building blocks of N-Acetyl-Glucosamine (GlcNAc) and N-Acetyl-muramic acid (MurNAc) upon which are attached pentapeptides, of usual sequence L-Ala – D-Glu – L-Lys – D-Ala – D-Ala. The precursors are synthesised in the cytoplasm, and both glucosidic and peptidic parts are attached by transglycosylases. The resulting product, a subunit of the cell wall, is anchored in the membrane through a C(55)-phospholipid (lipid A intermediate). The intermediate product then switches to the extra-cellular space, and transpeptidases ensure the mechanical cohesion of the peptidoglycan by crosslinking the pentapeptides. New subunits then come to grow the peptidoglycan layer (Figure 3). The large family of β-lactams (including penicillins, cephalosporins, monobactams, penems), as well as glycopeptides (vancomycin, teicoplanin) act by blocking some stage of the synthesis of the bacterial cell wall (or murein). B-lactams, with their conserved, eponym

chemical core (2-azetidinone), bind to cytoplasmic targets, initially called Penicillin-Binding Proteins (PBPs), which are enzymes (transpeptidases) that catalyse the last stage of cross-linking. Glycopeptides interfere with the activity of the transglycosylases by creating hydrogen bonds with the terminal D-Ala–D-Ala moieties of the MurNAc / GlcNAc – peptides, therefore avoiding the formation of the cell wall's backbone structure.

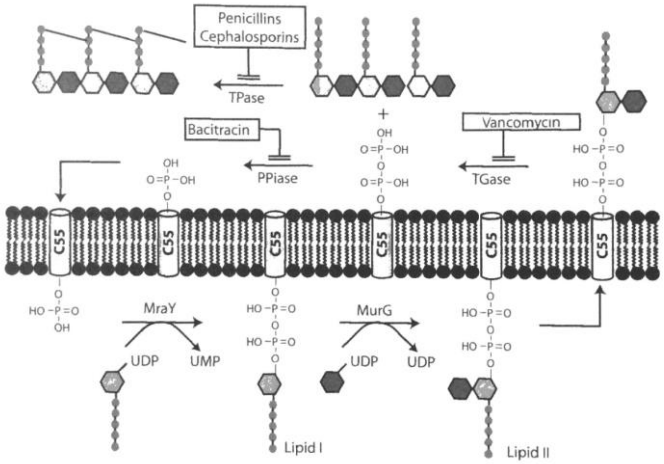

Figure 3. The cycle of the peptidoglycan assembly and inhibition of some stages by antibiotics. TGase:transglycosylase; TPase: transpeptidase; PPiase:pyrophosphatase. From [19].

Fosfomycin also acts on the murein synthesis pathway, although at one of the earliest stages, by inhibiting MurA, the first enzyme to assemble the MurNAc pentapeptide.

Insert 1. Pharmacodynamics of antibiotics acting against the cell wall synthesis.

Action on nucleic acid synthesis

Key to the DNA replication process are two families of enzymes, the DNA gyrases, ATP-dependent inhibitors of the supercoiling of the DNA strands, and the topoisomerases IV, whose role is to decatenate the DNA replicons. Among the fluoroquinolones family, sparfloxacin interacts with the gyrase, whereas ciprofloxacin, norfloxacin or levofloxacin interact with the topoisomerase IV, by covalent enzymatic links, preventing their essential actions and releasing a cleaved DNA. Of note, rifampicin (and rifabutin) also act at the nucleic acid level by inhibiting the DNA-dependent RNA polymerase.

Insert 2. Pharmacodynamics of antibiotics acting againt nucleic acid synthesis.

Action on protein synthesis

Involved in the physiological synthesis of proteins are a messenger RNA (mRNA) derived from the chromosomally-encoded DNA, the ribosome, an assembly machinery composed of a 50S and a 30S subunits and transfer RNAs (tRNAs) loaded with their amino-acid equivalent. When the amino-acyl tRNA (AA-tRNA) whose anticodon sequence is complementary to the mRNA codon is charged onto the acceptor site, a peptidyl transferase catalyses the peptide bond formation; the nascent peptidic chain is then translocated to the elongation site, leaving the acceptor and donor sites free to load the next amino-acyl tRNA, and elongation of the chain continues until the terminator codon is exposed.
Various antibiotics act on different stages of the protein synthesis. Interacting with the 30S subunit, aminoglycosides (streptomycin, tobramycin, gentamycin, amikacin) form a hydrogen-bonding network with the nucleic acid constituent of the 30S unit, the 16S ribosomal RNA (rRNA), especially in the acceptor site for amino-acid tRNA binding, resulting in mistranslated and misfolded proteins. The tetracyclines family (tetracycline, minocycline, doxycycline, tigecycline) also have their major binding site with the 16S rRNA, where they form a complex with divalent cations (Mg^{2+}), hence blocking the incorporation of incoming AA-tRNAs and stopping the peptide chain elongation. Still interacting with the 30S subunit is the oxazolidinones family (linezolid), which acts as a competitive inhibitor of peptidyltransferases. Interacting with the 50S subunit, lincosamides (clindamycin) as well as chloramphenicol prevent the formation of the peptide bond by inhibiting the peptidyl-transferases. Macrolides (erythromycin, roxithromycin, telithromycin), through three main structural elements (notably a macrolactone and a desosamine) can form up to seven hydrogen bonds with the structural 23S rRNA (key to the interaction is the adenine in position 2058, A_{2058}).

Insert 3. Pharmacodynamics of antibiotics acting against protein synthesis.

Action on other cell metabolic pathways

The bacterial synthesis of folic acid requires a major building block, the *para*-aminobenzoic acid (PABA), that the sulfonamides structurally mimic to take its place in the synthesis cycle and deprive the bacterium of this vital component. Trimethoprim, on the other hand, inhibits the dihydrofolate reductase, an enzyme that reduces the dihydrofolate (DHF) into tetrahydrofolate (THF), which is a precursor key to the thymidine synthesis pathway (therefore indirectly interfering with DNA synthesis).

Insert 4. Pharmacodynamics of antibiotics acting on other metabolic pathways.

APPENDIX 4.

Mechanisms of resistance

Antimicrobial agents	Mode of action	Resistance mechanisms
β lactams	Cell wall synthesis, cell division	β lactamase, altered penicillin binding proteins
Glycopeptides (azoles, cycloserine)	Cell wall division	Blocking of drug access to pentapeptide
Aminoglycosides (spectinomycin)	Inhibit protein synthesis (bind to 30S ribosome)	Enzymatic inactivation, altered target, impermeability
Macrolides	Inhibit protein synthesis (bind to 50S ribosome)	Altered target, enzymatic inactivation
Tetracyclines	Inhibit protein synthesis (affect t-RNA binding to 30S)	Efflux, altered target, impermeability, enzymatic inactivation
Chloramphenicol (lincosamides, streptogramin)	Inhibit protein synthesis (bind to 50S ribosome)	Enzymatic inactivation, impermeability
Quinolones	Replication: inhibit DNA gyrase	Altered target enzymes, impermeability
Rifampin	Transcription: inhibit DNA dependent RNA polymerase	Altered target enzymes, impermeability
Sulfonamides	Folic acid synthesis	Altered target
Trimethoprim	Folic acid synthesis	Altered target, impermeability

Table 4. Resistance solutions developed to the main classes of antibiotics. From [9]

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APPENDIX 5.

Surveillance programmes

Programme	Geography	Reference Body & Year
EARS-Net (European Antimicrobial Resistance Surveillance Network)	Europe	European Centre for Disease Prevention and Control (2010)
STRAMA (Swedish Strategic Programme against Antibiotic Resistance)	Sweden	SWEDRES (2009)
CIPARS (Canadian Integrated Program for Antimicrobial Resistance Surveillance)	Canada	Government of Canada (2010)
NARMS (National Antimicrobial Resistance Monitoring System)	USA	Centers for Disease Control and Prevention (2010)
DANMAP (Dansh Integrated Antimicrobial Resistance Monitoroing and Research Programme)	Denmark	DANMAP (2009)
ABC (Active Bacterial Core Surveillance)	USA	Centers for Disease Control and Prevention (2009)

Table 5. Examples of current governmental or institutional surveillance programmes.

Programme	Geography	Reference
SMART (The Study for Monitoring Antimicrobial Resistance Trends)	Global	Hsueh et al [10]
SENTRY	Global	Gales et al [11]
BSAC (British Society for Antimicrobial Chemotherapy)	United Kingdom	Morrissey et al [12]
T.E.S.T. (Tigecycline Evaluation Surveillance Test)	Global	Wang & Dowzicky [13]

Table 6. Examples of current industry-led surveillance programmes.

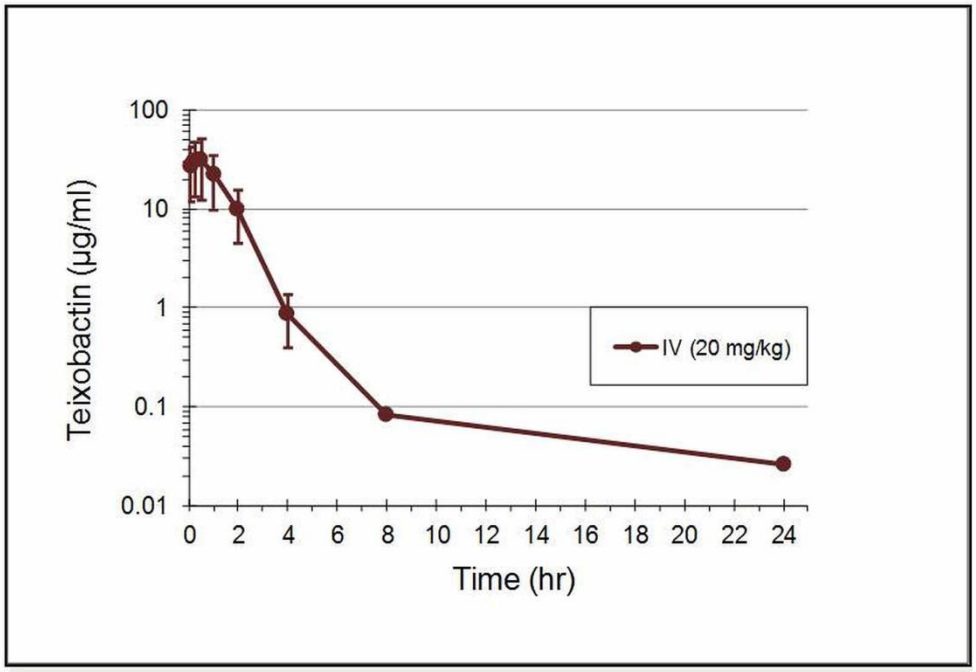
Global actions to combat antibiotic resistance

Figure 4. Proposed actions to limit spread of resistance and promote a rational use of antibiotics. From the WHO World Antibiotic Awareness Week [14].

APPENDIX 6.

Teixobactin

a



b

PK parameter	Definition	Value
C0 (µg/mL)	Initial concentration	27.2
AUC to Last (µg-hr/mL)	Area Under Curve to last time point	57.8
t1/2 (hr)	Half life	4.7
Total CL (mL/hr)	Clearance	6.9
Total CL (mL/min/kg)	Clearance	5.8
V (mL)	Volume of Distribution	47
Vss (mL)	Volume of Distribution at steady state	9.7
MRTINF (hr)	Mean residence time	1.4
Last Time point (hr)	–	24

Figure 5. Pharmacokinetics of teixobactin. a: evolution of the mean plasmatic concentrations of teixobactin (+ standard deviation) after a single IV injection in mice (20 mg/kg); b: additional pharmacokinetics parameters. From [15].

APPENDIX 7.

The organisation of the antibiotic industry

Firm name	Country
ABAC Therapeutics	ES
Abgentis	UK
Absynth Biologics	UK
Adenium Biotech	DK
AiCuris	DE
Alaxia Pharma	FR
Allecra	DE
Antabio	FR
Arsanis Biosciences	AT
Auspherix	UK
Basilea Pharmaceutica	CH
BioFilm Control	FR
Bioversys	CH
Biovertis	DE
Cantab Anti-infectives	UK
Da Volterra	FR
Debiopharm Group	CH
Deinobiotics	FR
Destiny Pharma	UK
Discuva	UK
Eligo Biosciences	FR
e-Therapeutics	UK
Evotec	UK
FAB Pharma	FR
Fundacion MEDINA	ES
Helperby	UK
Lamellar Biomedical	UK
MaaT Pharma	FR
Madam Therapeutics	NL
MGB Biopharma	UK
Motif Bio	UK
Mutabilis	FR
Nabriva Therapeutics	AT
Naicons	IT
Northern Therapeutics	FI

Nosopharm	FR
NovaBiotics	UK
Novacta	UK
Pherecydes Pharma	FR
Phico Therapeutics	UK
Polyphor	CH
Redx Pharma	UK
Summit PLC	UK
Synamp Pharmaceuticals	NL
VibioSphen	FR

Table 7. List of European biotech companies active in the antibacterial field as of April 2016 [16].

Firm name	**State**
Achaogen	CA
Aequor	CA
Agile Sciences	NC
AmpliPhi Biosciences	CA
Aridis Pharmaceuticals	CA
Cardeas Pharma	WA
Cellceutix	MA
Cempra Pharmaceuticals	NC
Crestone	CO
Entasis Therapeutics	MA
Macrolide Pharmaceuticals	MA
Melinta Therapeutics	IL
Microbion Corporation	MT
MicuRx Pharmaceuticals	CA (+China)
NovaDigm Therapeutics	ND
NovoBiotic Pharmaceuticals	MA
Paratek Pharmaceuticals	MA
Spero Therapeutics	MA
Symberix	NC
Tetraphase Pharmaceuticals	MA
Theravance Biopharma	CA
Trana Discovery	NC
VenatoRx Pharmaceuticals	PA

Table 8. List of US biotech companies active in the antibacterial field (as of 2016).

APPENDIX 8.

ACB's technical appendix and model outputs (extracts)

Materials & Method

The model was built in Excel (Microsoft Office Excel 2007), copies are available upon request. Several cases have been built to assess the robustness of the results. The base case scenario is described in detail here. The first part gathers assumptions and input cells, provided in Table 2 and Appendix 3, Tables 3-5. Nine inputs have been defined and the values have been documented in the literature if available; otherwise the data represent the author's own estimates. The second part actually models the investment cycles, with the cash flow from the amount raised by investors to the money available at the end of the cycle.

Each investment cycle starts with an estimate of the money raised through the bond. To capture the maximum of granularity, and in the absence of a documented specific statistical distribution, each amount invested is a number whose probability of appearance is based on a Normal distribution (the closer to the mean, the higher the likelihood of appearance). Two types of inputs are allowed: a series simulation based on mean and standard deviation (user inputs), or based on chosen minima and maxima. The generation of the data series is based on the NORM.INV formula: in the first input case, mean and standard deviation, the function's arguments, are directly entered; in the second case, they are back-solved from the inputted minimum and maximum figures. Once the amount raised is determined, the financing cycle can begin. The next step calculates the money effectively available for the grants, obtained by subtracting the cumulated coupon payments during the lifetime of the bond (coupon = yield * duration * amount raised). The next step calculates the number of companies that can be funded, by dividing the money available by the minimum grant per company, with the limit of 4 companies funded. Then the grants are calculated: to capture the most volatility, these are random numbers generated between the minimum and maximum possible grants (user inputs). This is modelling the amount of money a company would ask for, but then a check if applied ensuring that this amount is granted only if it is less than the money available to the agency (columns '*Est.*' and 'Granted'). The distribution of grants is sequential, meaning that grants are distributed one after another, and as a consequence the last companies are less likely to get the total amount they required. The model then calculates if there is some money left after the distribution of the grants ('Extra money remaining').

Once the grants are distributed, the scientific part begins. The move to the next step in the model is weighted by the probability of success in the development of an antibiotic. The probability can be of two types, upon user's preference: either a point estimate (e.g., 15% probability of success) or a number chosen randomly in a range (e.g., probability of success ranging between 15 and 25%). Variability can also be captured at the company level, as the model allows for different probability types (point estimate or range) and different data inputs for each of the four companies.

The next stage, conditional on the successful launch of a novel antibiotic, is the PEC auction process. Once again, to increase variability, the money raised by the sale of the PEC has been assumed to follow a Normal distribution, with a user-defined mean and standard variation, or minima and maxima figures (similarly to the generation of the series for money raised by investors).

The last steps once the proceeds are collected and before the investment cycle closes involve repayments and money distribution. Any successful company is given a lump sum payment as a reward (user-defined), and the agency retains some of the proceeds as administrative fees (user-defined). Once these two figures are subtracted from the PEC sale results, the final figure shows how much benefit or loss (if no drug approved during the cycle) the agency is left with. If the number is negative, the next column alerts that the agency is in the red. Finally, the last column provides the cumulative result for several investment cycles.

Insert 5. The Antibiotic Corporate Bond: Model assumptions and methods.

Assumption	**Values**	**Ranges tested**
Money raised by investors	Values generated based on a Normal distribution* $\mathcal{N}(1000,200)$[1]	• Min 100 – Max 1,000 • $\mathcal{N}(1000,200)$
Service of the bond	Duration (years): 10[1] Yield: 3%[1]	15 -
Minimum grant per company	200[1]	100
Maximum grant per company	400[2]	500
Probability of success (per company)	Option 1: Probability value: 11.83%[3] Option 2: Probability range: 9.4 – 16%[4]	-
Amount raised by sale of PEC	Values generated based on a Normal distribution* $\mathcal{N}(4000,800)$[1]	• Min: 300 – Max: 5,000 • $\mathcal{N}(2000,600)$
Reward to successful company	1,750[1]	1,000
Agency administrative fees	5[2]	-

*Table 9. Main assumptions in the ACB model. *: complete number generation not detailed here. Data in $m. Notes: 1. Barclays estimates; 2. Author's estimates; 3. Source: [17] . 4. Source: ERG modelling assumptions and IMS data, from [18].*

Results

The model was run for 200 cycles with the base case numbers as per Table 2. On average, the money raised by investors was $1,004m, the money available to the agency was $703m, and the number of companies funded was 3. The grant amounts ranged from $110.4m (granted to the third company) to $306m (to the first company). The agency had some extra money in only 7.5% of the cases; this sense check confirms the ability of the model to correctly distribute the money. On average, the companies had a success rate for drug development of 9.4%, and where available, the PEC was sold on average for $4,076m. Furthermore, the agency ended in deficit in the majority of cases (61.5%), although when cumulating the profits/losses over the cycles, the model proved itself very beneficial in the long run: although in deficit for the four first cycles, it reached balances of $5bn at the 5th cycle, $10.6bn at the 10th cycle, and $22.5bn after the 20th cycle (with the model not allowing for profits to be re-invested).

Insert 6. Results from the ACB.

Sensitivity Analyses

The model was designed to try and answer questions about the feasibility of the ACB and provides flexibility to test different cases. Firstly, how many drugs would it help bring to market? In this simulation, 2 antibiotics have been approved in 10 years, or 5 on a 20-year horizon. The model does not take any scientific consideration into account though, and only assesses the results from financial incentives. Secondly, can the model be self-funded? This question is important due to the backing of the agency by governments in case of losses. With the current assumptions, the model was self-funding after five year, but the advantage is that it only needs one success to become financially self-sufficient and withstand several other setbacks, given the size of the proceeds gathered by the sale of the PEC. Further conditions, such as reimbursement to the government once the agency has the capacity to, could even lead to a neutral situation where every stakeholder is better off compared to the initial case.

Insert 7. Sensitivity analyses of the ACB model.

Internal Validity

Because the economic evaluation is based on a computer simulation, it has several limitations that may impact the robustness of the conclusion. These limitations are at two levels: they can concern either the design of the model or the numerical assumptions used. The scaffold of the ACB has been structured to ensure an easy and sequential flow, with each important step represented by a separate column. However, one of its limitations is the rigidity in the ordering of events: the money for coupons payment is set aside at the start of the cycle, for instance, and the sequential grant allocation system implies a "first come, first served" distribution. Another limitation arising from the structure of the model is the number of companies funded: the maximum number has to be set in advance because it determines the number of columns required. Nevertheless, the software allows creating a copy of the model in a separate tab where this assumption can easily be changed. A similar point is raised with the agency's net income at the end a cycle: by design, the profits, if any, are not reinvested across cycles, but here again, an additional column of code in a separate tab could address this limitation. On the other hand, one of the strengths of this structure is its ability to incorporate complex assumptions in a user-friendly manner: only the cell inputs at the top of the output or data sheets need to be changed. Complex series generation based on statistical distributions has been made possible, with the advantage of capturing more granularity. Turning to the discussion of the assumptions, there is a plethora of variations possible, and for such a model does not exist in reality, every single numerical step and assumption can be challenged based on one's particular views and forecasts.

Insert 8. Internal validity of the ACB model.

Data 1 - Money raised by investors (in millions)

Assumption: amount raised follows a Normal distribution; mean and standard deviation displayed below

1. Switch button:
- if use self-inputted mean and std dev, choose: 1
- if use self-inputted min and max, choose: 2

Switch: 1

mean	1000	1000
stdev	200	200

approximate range of amount raised:

min	400	40
max	1600	2000

2. Results

generated based on the formula: NORMINV(RAND(),C8,C9)

836.3338	974.8103	1006.581	1162.848	1205.699	784.4447
941.9651	1446.993	897.3271	895.3579	1557.731	1312.195
581.2138	1211.575	1200.36	838.9989	1165.645	1117.467
694.0054	1085.718	967.7858	1226.987	1036.144	923.1086
949.6496	645.7036	572.5925	1087.86	1135.01	1253.247
983.1139	897.1369	928.5685	1193.162	823.7409	643.9867
1315.734	674.5156	958.9696	1067.353	978.952	854.3484
1105.434	1095.387	900.6106	983.7145	1142.94	898.3858
1189.552	920.2752	1467.183	673.041	1065.049	876.3393
1058.028	1320.374	975.3121	730.18	1283.971	842.3421
839.2493	1201.588	1062.925	1002.973	1074.08	678.7737
790.9656	972.0821	958.8974	1309.848	1222.5	953.4894
1447.974	988.2241	778.7018	1250.156	1124.464	1141.401
742.2121	940.6013	797.7999	876.4356	922.4552	1077.695
917.3264	1125.606	1136.053	859.1277	616.3202	849.3083
764.9203	872.1783	787.7337	1019.953	1231.887	576.0811
1373.716	1323.55	1039.178	847.7903	766.0299	
708.407	938.1863	1110.243	902.8248	1154.357	
835.7048	820.9736	969.7384	942.4701	1210.882	
870.141	682.3634	891.9409	980.572	1197.489	
1340.939	939.9156	860.9968	997.7585	994.1034	
1400.563	1249.608	880.5879	1105.593	711.3637	
1198.718	1274.935	1003.001	843.7968	859.4335	
919.5877	1034.731	1410.879	819.2574	1037.931	
1176.289	1149.582	1121.089	911.1185	1043.36	
1186.685	937.6263	981.3737	1123.661	980.3301	
1047.814	1028.052	881.2182	980.723	1080.402	
1012.542	720.5537	1030.058	887.9796	1006.11	
1052.79	1214.412	818.9296	844.9977	421.3303	
780.8627	1174.993	1245.959	1107.007	1201.998	
792.1042	1155.784	840.6518	841.8037	1324.634	
1050.334	1144.11	1162.908	1008.71	949.5433	
908.5954	820.8639	956.2514	950.4455	896.5415	
788.1658	1125.01	957.1306	1066.921	1504.096	
1075.719	1301.008	1213.879	1377.462	1294.335	
1006.063	572.5434	909.6016	1138.261	726.9381	
974.8103	631.614	862.8514	1106.317	1179.107	

3. Visualisation: Histogram of data generated (check range of data selcted)

NOTE: Check if chart has all data selected

If the standard deviation is high the chart will not have taken all the data points

	Frequency
400	0
450	1
500	0
550	0
600	4
650	4
700	5
750	6
800	10
850	16
900	18
950	19
1000	22
1050	16
1100	14
1150	18
1200	13
1250	13
1300	5
1350	8
1400	2
1450	4
1500	1
1550	1
1600	1

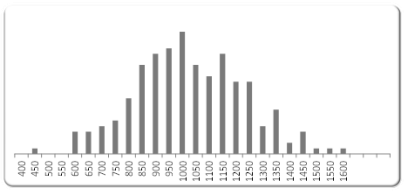


Figure 6. Details of the 'money raised by investors' data set.

Data 2 - Money raised by auction sale of PEC (in millions)

Assumption: amount raised follows a Normal distribution; mean and standard deviation displayed below

1. Switch button:
- if use self-inputted mean and std dev, choose: 1
- if use self-inputted min and max, choose: 2

Switch: 1

mean	4000	4000
stdev	800	800

approximate range of amount raised:

min	1600	3000
max	6400	5000

2. Results

generated based on the formula: NORM.INV(RAND(),C8,C9)

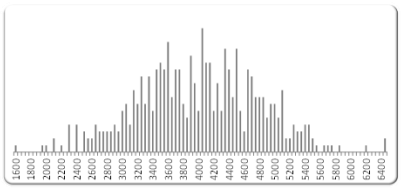
3851.55
4618.30
3430.72
4040.71
3905.39
4339.31
3546.78
3713.93
4267.03
3017.86
3854.13
4644.22
4452.08
3657.19
3230.96
5403.80
2392.84
4727.17
3467.52
4406.61
3194.63
4358.36
3927.67
5389.92
4250.58
3671.13
2681.47
5220.15
4873.82
2842.16
4826.20
5135.60
5243.64
2376.18
2367.40
3545.77
4228.03
3908.51

3. Visualisation: Histogram of data generated (check range of data selcted)

NOTE: Check if chart has all data selected (the default selects data for x={I15:I70} and data for y={K15:K70}

If the standard deviation is high the chart will not have taken all the data points

1600	1
1650	0
1700	0
1750	0
1800	0
1850	0
1900	0
1950	1
2000	1
2050	0
2100	2
2150	0
2200	1
2250	0
2300	4
2350	0
2400	4
2450	0
2500	3
2550	2
2600	2
2650	4
2700	3
2750	3
2800	3
2850	3
2900	4
2950	3
3000	6
3050	7
3100	4
3150	9
3200	7
3250	11
3300	7
3350	11
3400	6
3450	12



4. Data for model

Company 1	Company 2	Company 3	Company 4
3851.55	2377.715	3936.637	4091.705
4618.30	3756.036	3916.002	4731.389
3430.72	3498.262	4347.974	3480.417
4040.71	4306.691	2990.578	5495.278
3905.39	5465.123	4253.386	3724.713
4339.31	4206.176	5082.299	3873.264
3546.78	4019.37	3156.248	3285.953
3713.93	2770.62	3793.014	4894.755
4267.03	4208.803	4508.909	3621.472
3017.86	4633.832	5288.104	6097.581
3854.13	4046.658	2173.593	3774.366
4644.22	3582.478	4208.723	4745.443
4452.08	3406.973	4432.393	3020.668
3657.19	4256.878	4252.949	4329.248
3230.96	4326.879	3941.314	4977.247
5403.80	3995.396	4796.558	4049.353
2392.84	3435.225	4742.124	3556.592
4727.17	3637.401	4147.677	4522.961
3467.52	4089.707	4807.032	4363.373
4406.61	4209.86	4232.603	1895
3194.63	4410.011	4009.34	6176.103
4358.36	3110.103	4268.665	3714.125
3927.67	4548.948	5923.884	3136.303
5389.92	3775.54	4668.384	5081.174
4250.58	1563.398	3944.618	3160.912
3671.13	4998.17	5871.246	4203.287
2681.47	3984.022	3750.029	3115.933
5220.15	2726.225	5226.566	4395.188
4873.82	4479.691	3223.307	3506.771
2842.16	4500.106	3549.288	5621.05
4826.20	4024.988	4448.068	5505.538
5135.60	4462.377	3951.749	5497.24
5243.64	3691.775	5021.032	4995.815
2376.18	4724.298	4077.367	3546.334
2367.40	3130.991	5326.861	4166.317
3545.77	3805.728	3984.924	4655.285
4228.03	3359.351	4613.626	3789.029
3908.51	4690.614	4457.023	3974.892

Figure 7. Details of the 'money raised by auction sale of PEC' data set.

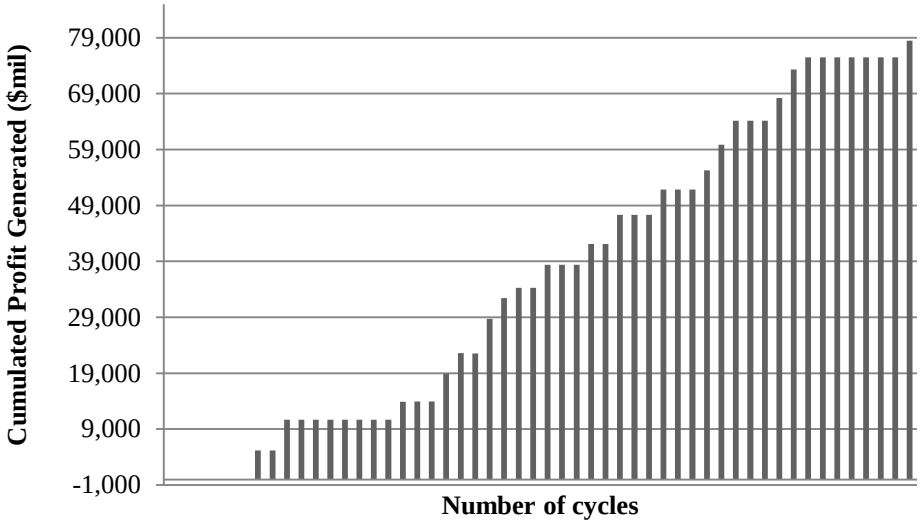


Figure 8. Cumulative amount of money controlled by the ACB agency (50 first cycles)

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Université de Lille 2 DROIT ET SANTÉ

Faculté des Sciences Pharmaceutiques et Biologiques de Lille

FACULTE DES SCIENCES PHARMACEUTIQUES ET BIOLOGIQUES

DECISION D'AUTORISATION DE SOUTENANCE

Le Doyen de la Faculté des Sciences Pharmaceutiques et Biologiques de LILLE,

Vu la loi d'orientation de l'Enseignement Supérieur,

Vu l'arrêté du 17 JUILLET 1987 et notamment ses articles 28 et 29,

Vu la décision du Président de l'Université en date du 14 mai 2012 relative aux délégations de signature :

DECIDE

ARTICLE 1 : **Madame Alice RENARD** est *autorisée à soutenir une thèse en vue de l'obtention du Diplôme d'Etat de Docteur en Pharmacie sur le sujet suivant :*

THE EVOLVING LANDSCAPE OF THE ANTIBIOTIC INDUSTRY : NOVEL SCIENTIFIC APPROACHES, CHANGING ECONOMIC FRAMEWORKS AND REGULATORY MEASURES

ARTICLE 2 : *La soutenance aura lieu le* ***vendredi 17 juin 2016***

à 18h15

Amphithéâtre Pauling

ARTICLE 3 : *Le jury sera composé ainsi qu'il suit :*

PRESIDENT : ***Monsieur le Professeur André TARTAR***
Faculté des Sciences Pharmaceutiques et Biologiques LILLE
UNIVERSITE LILLE 2

ASSESSEURS : ***Monsieur le Professeur Nicolas WILLAND***
Faculté des Sciences Pharmaceutiques et Biologiques LILLE
UNIVERSITE LILLE 2

MEMBRE(S) EXTERIEUR(S) : ***Madame Nicole BARBE***
Responsable Affaires Réglementaires

LILLE, le 17 mai 2016

LE *DOYEN,*

Faculté des Sciences Pharmaceutiques et Biologiques de Lille Université de Lille

D. CUNY

Université de Lille 2
FACULTE DES SCIENCES PHARMACEUTIQUES ET BIOLOGIQUES DE LILLE
DIPLOME D'ETAT DE DOCTEUR EN PHARMACIE
Année Universitaire 2015/2016

Nom : RENARD
Prénom : ALICE

Titre de la thèse :

Is bacterial resistance currently driving a paradigm shift in the antibiotic industry? Novel scientific approaches, changing economic frameworks and specific regulatory measures to fix antibiotic innovation and drug development

-

La résistance aux antibiotiques, un phénomène actuellement responsable d'un changement de paradigme dans l'industrie antibiotique? Apport des nouvelles techniques scientifiques, des modèles économiques et des mesures réglementaires spécifiques pour relancer l'innovation et le développement des antibiotiques

Mots-clés : Résistance bactérienne – antibiotiques – résistance : épidémiologie – modèles économiques – réglementation des antibiotiques – incitations économiques et réglementaires pour la découverte de nouveaux antibiotiques

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Résumé :
La situation de la résistance bactérienne aux antibiotiques dresse en 2016 un tableau qui alarme les politiques et dont les impacts en santé et économiques sont conséquents. Face à des cibles toujours plus ingénieuses dans leurs mécanismes de résistance, les bactéries, les armes que représentent les antibiotiques sont en passe de perdre l'avantage si l'innovation et l'intérêt que leur portent les entreprises pharmaceutiques ne sont pas renouvelés. Trois principaux défis à ce but sont identifiés : les capacités scientifiques, les modèles économiques et les mesures réglementaires. Les solutions requises sont parfois trop novatrices pour s'inscrire dans la continuité du développement traditionnel des antibiotiques, mais un tel changement de paradigme pour relancer l'innovation dans les thérapies antibiotiques ne serait-il pas dans l'intérêt de santé publique ?

<u>Membres du jury</u> :

Président : M. Tartar André, Professeur des Universités, Faculté des Sciences Pharmaceutiques et Biologiques de Lille

Assesseur(s) : M. Willand Nicolas, Professeur des Universités, Faculté des Sciences Pharmaceutiques et Biologiques de Lille

Membre(s) extérieur(s) : Mme. Barbe Nicole, Responsable Affaires Réglementaires, Laboratoire Janssen-Cilag, Issy-les-Moulineaux