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TACKLING ANTIMICROBIAL RESISTANCE: THE ROLE OF SYNTHETIC BIOLOGY

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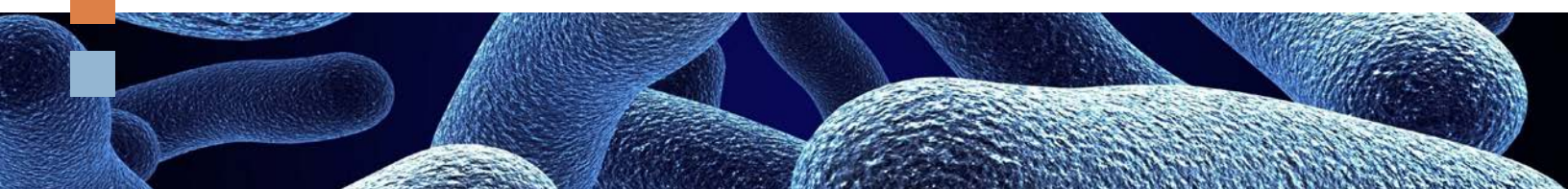
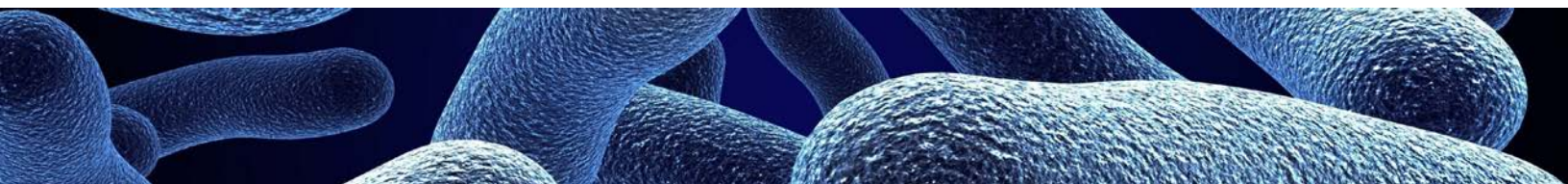


"The future of humanity and microbes likely will unfold as episodes of a suspense thriller that could be titled 'Our Wits Versus Their Genes'"
- Joshua Lederberg, Nobel laureate



PREFACE

This report has been written during a six-month internship at the Dutch Institute for Public Health and the Environment (RIVM), as part of the specialization 'Science, Business and Policy' of the master 'Biomedical Sciences' at the University of Groningen (RUG).

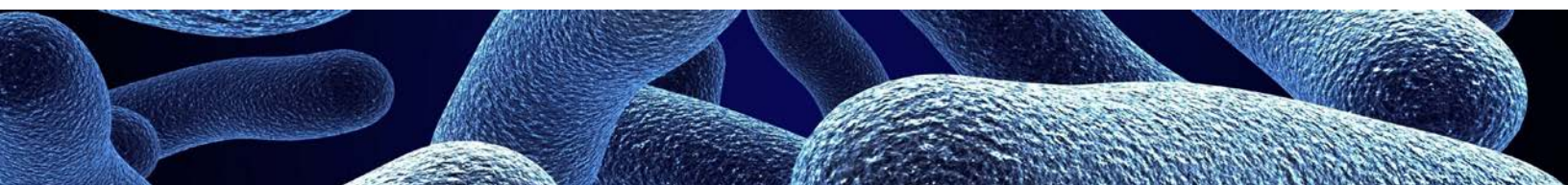
I have always had an interest in both the biomedical sciences and policy. For me, the RIVM seemed to be the most suitable organization to put these interests into practice. Luckily, I was not wrong. When offered this internship, the field of synthetic biology was new to me, and I only had some basic knowledge on antimicrobial resistance. However, both subjects seemed to be complex and very interesting, making it easy to choose this particular internship. The fact that an SBP alumni was going to be one of my supervisors on this project, was also contributing greatly to its attractiveness. Many times, the excitement of discovering both subjects had to be restrained, in order for me not to wonder off into articles or reviews that were not focused on the AMR/synbio combination.

The thing that I probably appreciated the most in this internship, was the constant reminder that it was my project to lead. Both Jaco and Korienke, my direct supervisors at the RIVM, have always stimulated me to be independent and take initiative, while also offering support whenever necessary. They always had the time to answer my questions, offer ideas or give me feedback, for which I am very thankful. I also want to thank my supervisors in Groningen, Maarten van den Nieuwenhof and Oscar Kuipers. Maarten, for providing reflections both on the project and my personal development. Oscar, for his quality input on the sometimes confusing world of synthetic biology.

Besides my supervisors, there are many others to thank for making my internship truly valuable and enjoying. Cécile van der Vlugt, for offering clear and helpful explanations of the scientific part of my project, as well as providing feedback whenever necessary. At the CIb, I would like to thank Mariken van der Lubben, for her time to ask critical questions and provide me with feedback on the world of AMR. Pita Spruijt, for quality input on many methodological issues, and Mark van Passel, for his help structuring my project at the start of my internship.

I would also like to acknowledge several other people who offered their time and support throughout this process. First, Heddy de Wijs, for her enthusiasm and inspiring words. Second, Andre Krom, for providing a fresh outlook on various parts of my project. Third, Dirk Stemerding and Virgil Rerimassie from the Rathenau Institute for inviting me to offer input on their stakeholderdialogue on AMR and synbio, and their enthusiasm and inspiration. Fourth, I would like to thank all the people at GBV for making my time very enjoyable by chats in the hallway, a pubquiz and of course, awarding my cheesecake with the first prize in 'Heel VSP bakt'. Finally, I would like to thank both family and friends for support, and the other students at the RUG, and in particular Matthijs, for their feedback.

Kind regards and happy reading,
Hanneke van Mierlo
Bilthoven, 04-07-2016



EXECUTIVE SUMMARY

Antimicrobial resistance (AMR), the resistance of microbes towards medicine, has been stated to be one of the most significant challenges to the health care sector in the 21st century and one of the biggest global health problems today. It is a complex and multifaceted problem which requires many concurrent solutions – one of them is the development of new antibiotics, alternatives to antibiotics and appropriate diagnostics. This report has focused on this limited pipeline, for which innovation is required.

Synthetic biology (synbio) is one of the research areas that could aid in this need for innovation. It aims at designing biological systems, components and processes, which do not already exist in nature, together with the re-design of existing biological systems. This report shows that synbio can offer novel approaches towards the development of both new antibiotics, alternatives to antibiotics, and diagnostics. Developments such as the creation of new-to-nature molecules, the engineering of bacteriophages and the development of paper-based diagnostics could play a role. Most of these developments however, are still in their proof-of-principle phase and still remain far from licensed and clinically approved products. In addition, the synbio/AMR field in the Netherlands is relatively small.

It is however of importance to closely monitor these developments, not only on national level, but also on international level, in order to identify barriers (for example regulatory barriers) in further development and production and timely remove these, while safeguarding risks and considering societal and ethical issues. The RIVM, more specifically the Centre for Safety of Substances (VSP), has the potential to do so, as it aligns with their current activities in signaling and advising the Ministry of Infrastructure and the Environment (I&M) on new developments within modern biotechnology. This knowledge can then be shared with the Direction Medicines and Medical Technology of the Ministry of Health, Welfare and the Environment (VWS), as they are responsible to remain up-to-date with the current AMR developments and can aid in the removal of these types of regulatory barriers. In addition, as many stakeholders within the antibiotic innovation pipeline were unfamiliar with the AMR/synbio developments, they could benefit from this information as well. This report can already contribute to this goal. As VWS is not the main commissioner of VSP, it can be beneficial to create a better cooperation with the Centre for Infectious Diseases (CIb).

The Dutch government, mainly the Ministry of VWS, can play a bigger role in stimulating AMR innovation in general, and additional research on the various viewpoints of stakeholders in this process on the role of the government herein is necessary. However, taking into account the considerations that have already been made, it is recommended that VWS facilitates an external network organization, financially independent from the market, which can execute various functions. These include the facilitation of networks throughout the antibiotic innovation pipeline, matching the expectation of end-users and scientists (health need and research), and staying up-to-date with research and development in both public and private settings. The RIVM and the Direction Medicines and Medical Technology could cooperate towards the latter goal. This way, a direct involvement of the government in the private sector can be avoided and both public interests and public goals could be safeguarded.

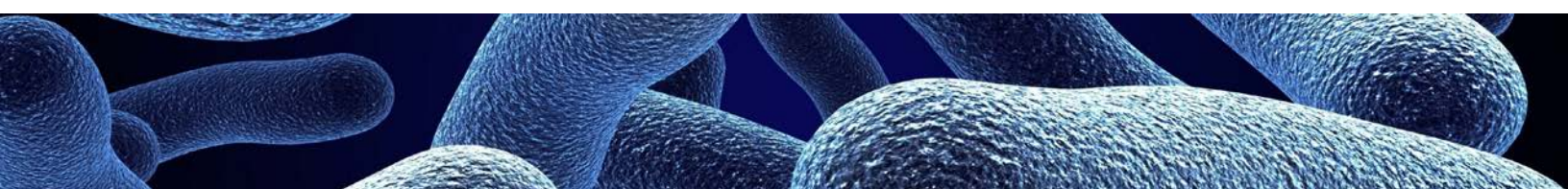
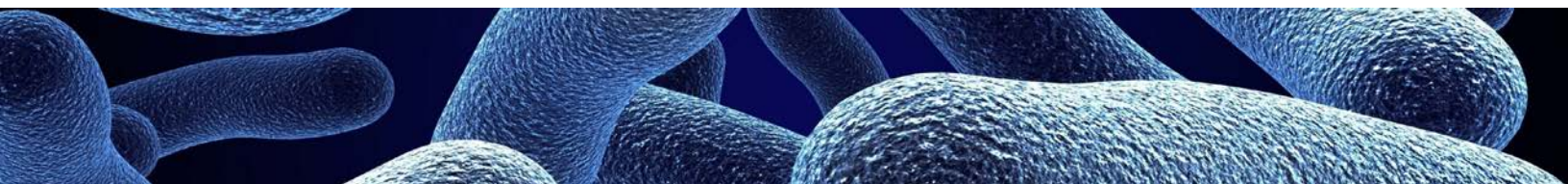
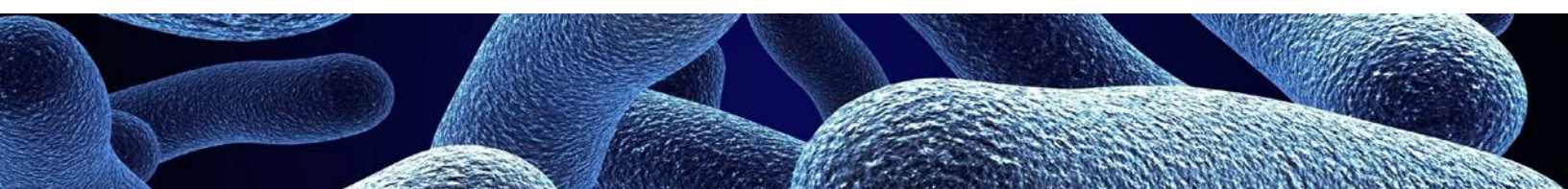
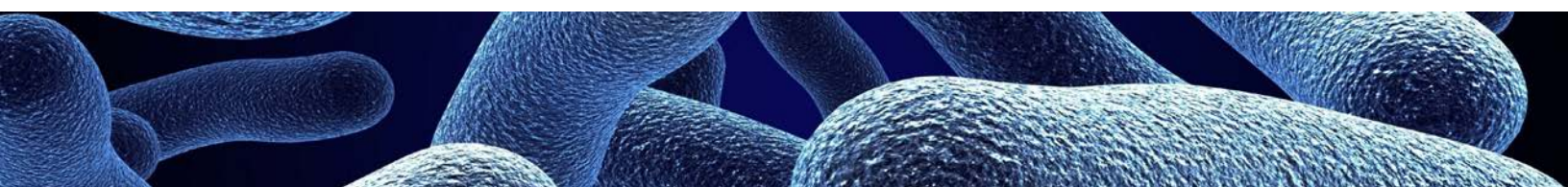


TABLE OF CONTENTS







LIST OF ABBREVIATIONS

ABR: Antibacterial resistance

AMPs: Antimicrobial peptides

AMR: Antimicrobial resistance

BCGs: Biosynthetic gene clusters

CDC: US Centers for Disease Control and Prevention

CIb: Centre for Infectious Disease Control

EC: European Commission

GMT: Direction of Medicines and Medical Technology.

GUARD: Global Union for Antibiotics Research and Development

HTS: High-throughput screening

iGEM: Internationally Genetically Engineered Machine

IMI: Innovative Medicines Initiative

I&M: Dutch Ministry of Infrastructure and the Environment

JPIAMR: Joint Programming Initiative on Antimicrobial Resistance

LSE Health: London School of Economics' health research centre

MIT: Massachusetts Institute of Technology

NCOH: Netherlands Center for One Health

NRPs: Non-ribosomal peptides

R&D: Research and development

RIVM: National Institute for Public Health and the Environment

SBP: Science, Business and Policy Master program at the University of Groningen

SMEs: Small and medium enterprises

Synbio: Synthetic biology

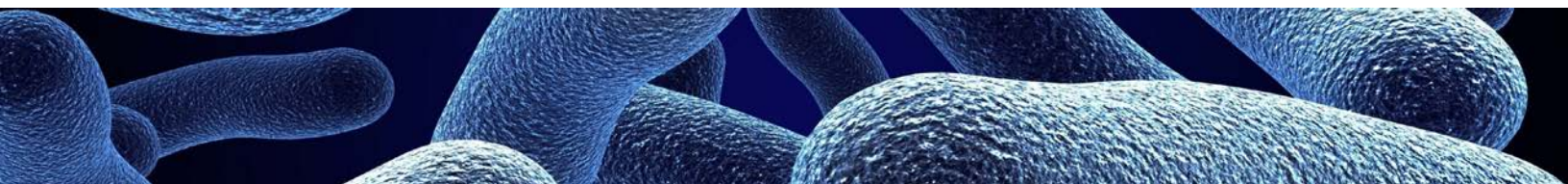
TATFAR: Transatlantic Taskforce on Antimicrobial Resistance

VSP: Centre for Safety of Substances and Products

VWS: Dutch Ministry of Health, Welfare and Sport

WHO: World Health Organization

ZonMw: Netherlands Organization for Health Research and Development



CHAPTER 1 - PROJECT INTRODUCTION

1.1. BACKGROUND

The National Institute for Public Health and the Environment (RIVM) is an organization with various tasks and responsibilities. Different centers have different key expertises and give advice to different government authorities. While some centers have a more executory function towards tackling specific public health or environmental problems, others have a more signaling function when it comes to new (bio) technological developments. Among these are developments that could provide society with several solutions towards tackling these public health or environmental problems. It is therefore of importance that links between different key expertises are made, and thus between different centers.

One such a link is the use of synthetic biology towards tackling antimicrobial resistance (AMR). AMR is the resistance of microbes towards the drugs that we use against them. Although antimicrobial resistance is a multifaceted and complex problem, which requires several solutions, one of them is the development of new antibiotics or alternatives to antibiotics, or diagnostics. On the other hand, synthetic biology is an emerging research area, which aims at designing biological systems, components and processes, which do not already exist in nature, together with the re-design of existing biological systems. Its advances could provide the world with new approaches towards tackling various global environmental and medical problems, amongst which the creation of organisms that can product clean fuels, or that can recognize and destroy tumors. In addition, it could provide solutions towards the development of new therapeutics and diagnostics to tackle AMR. It is important for the RIVM to stay up-to-date with these developments, to gain insight in the chances, barriers, risks and impact that come along with them.

1.2. OBJECTIVES

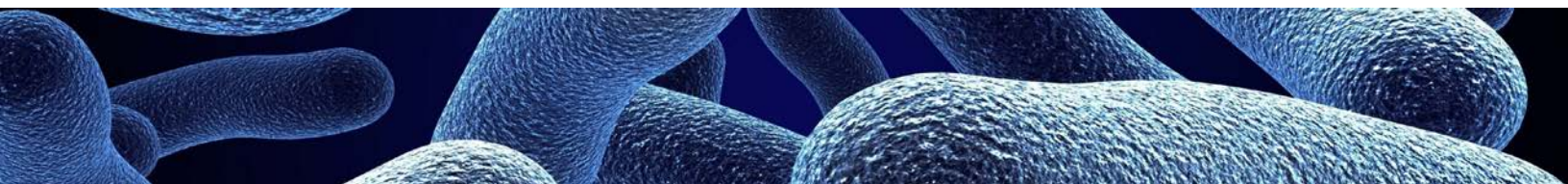
The report has two objectives, which are reflected in the two different chapters.

The first objective is to create an assessment of the developments in synthetic biology that are relevant for antimicrobial resistance. This includes both new antibiotics, alternatives to antibiotics, and diagnostics.

The second objective is to map some considerations that can be made by the government (mainly the ministry of Health, Welfare and Sports) on their role in (synbio) innovation in AMR.

1.3. METHODS

To create the assessment, an extensive literature study has been done, as well as exploratory interviews with different scientists in the synbio/AMR field. New developments were identified scanning scientific literature and various news sources. Scientific reviews were used as a starting point, after which further literature was found using keywords used in these reviews.



To map the considerations, an assessment of the different stakeholders involved was made. These were identified based on their involvement in a self-made simple linear model of the antibiotic innovation pipeline. Three types of interviews were held with these stakeholders: exploratory interviews, semi-structured interviews and an interview for critical reflection. This process in explained in more detail in chapter 6 and a full list of interview participants can be found in Appendix II.1. In addition, a structured questionnaire with both open and closed questions was used to gather the opinion of mainly scientists, present on the Antibiotics Now! Symposium, on the viability of the current developments on new antibiotics and alternatives to antibiotics. The questionnaire can be found in Appendix IV, and a summary of the Antibiotic Now! Symposium can be found in Appendix III.3.
To place these results into context, the current policy towards innovation in AMR has been assessed. For this, policy documents on current and previous AMR policy on national, European and global level have been studied.

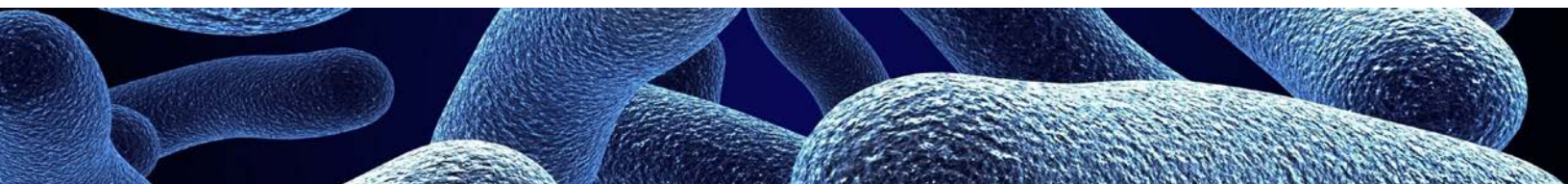
1.4. READING GUIDE

As stated above, this report is divided into two parts.

Part one will first provide the reader with a general description of both synthetic biology and antimicrobial resistance. For the latter subject, the difference between Gram-positive and Gram-negative bacteria will be explained, and a short history on antibiotic discovery will be given. This provides the reader with the basic information necessary in order to understand the following chapter. A more extensive overview on the type of bacteria that we should worry most about, the working mechanisms and classification of our current antibiotics and how the bacteria become resistant towards these antibiotics, can be found in Appendix I.1 (Knowledge Document).

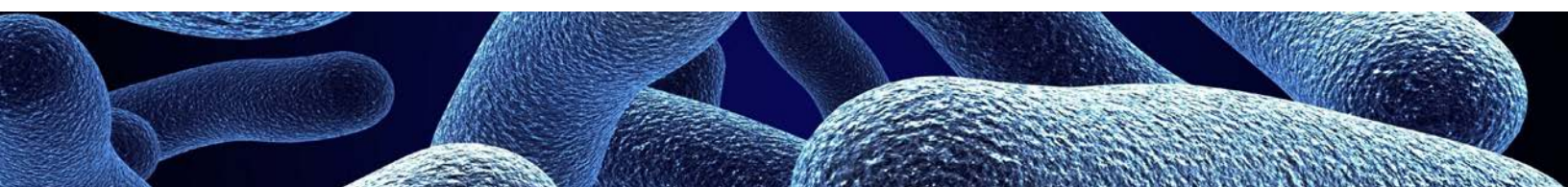
Next, the developments within synthetic biology that could influence AMR will be described. This chapter will focus not only on the development of new molecules to serve as antibiotics, but also on synbio alternatives to our current antibiotics, and synbio diagnostics that could aid us in determining which bacteria is causing the infection. The chapter provides the reader with information on these developments mainly on a conceptual level (occasionally illustrating these concepts with examples). A more detailed overview can be obtained using this chapter together with the knowledge document in Appendix I.2.

Part two will first provide the reader with an outline of the aim and approach of this part. The reader is taken through a simplified linear model of the (public) antibiotic innovation pipeline, and is given explanations of the processes involved. To create insight and to be able to interpret the results of the questionnaire and interviews, the current AMR policy framework is given, including AMR funding policy. Finally, the results of the interviews and questionnaire will be discussed and concluded, and recommendations will be given.



1.5. SCOPE

- Antimicrobial resistance (AMR) encompasses resistance to drugs that treat infections caused by bacteria, as well as other microbes, such as fungi, parasites or viruses. Although the term AMR will be used most frequently in this advice, it is mainly focused on the resistance that occurs in bacteria towards antibiotics. The reason for this is the greater incidence of serious pathogenic bacterial infections. In addition, in the Netherlands, the more widely used term that is used is antibacterial resistance (ABR). It is also recognized that antibacterials and antibiotics have different medical definitions. However, these terms are used interchangeably in this report, as they are generally used as synonyms in the relevant literature.
- There is a focus in AMR on the so-called 'One Health' approach, which includes all areas where AMR has an impact: humans, animals, the environment and food. Although its importance is acknowledged, the focus in this report will be on synbio developments focused on human health. The developments in synbio that could contribute to the problem in other areas are certainly interesting, but due to time constraints, it was not possible to include them all.
- The developments within synbio that could be of influence to AMR that are mentioned here, are mostly focused on applied research. There are other important synbio developments that focus more on fundamental research (such as the development of resistance), but these are not included in this paper.
- A thorough literature search has been performed, after which these results were submitted to two synthetic biologists to check for apparent hiatuses. However, due to the sheer amount of scientific papers, not all papers were included due to limited space and time constraints.



CHAPTER 2 -THE NATIONAL INSTITUTE FOR PUBLIC HEALTH AND THE ENVIRONMENT (RIVM)

2.1 THE RIVM – A GENERAL DESCRIPTION

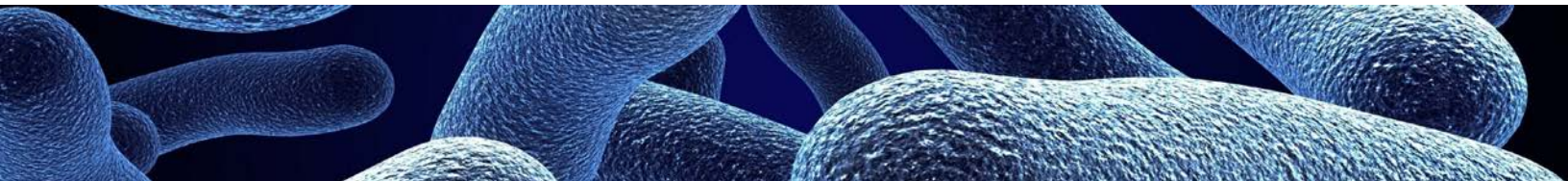
The Dutch National Institute for Public Health and the Environment (RIVM) performs independent research and provides government authorities on all levels with policy advice. The RIVM collects and collates knowledge and information from various sources, national and international. This knowledge is applied by the RIVM, but is also placed at the disposal of policy makers, researchers, regulatory authorities and the general public [16].

In its role as a trusted advisor, the RIVM provides the government with impartial advice on various subjects, including infectious diseases, population screening, nutrition, pharmaceuticals, environment, sustainability and safety. This work is mainly commissioned by Dutch ministries and inspectorates. In addition, projects are also undertaken within international frameworks, such as the European Union and the United Nations [16]. Independence is being maintained in various ways, one of which is making their research results publically available in reports and on their website.

2.2. DIFFERENT CENTERS, DIFFERENT ROLES

The RIVM is a large organization, consisting out of approximately 1500 employees. The organization consists of three domains with specific knowledge and expertise. Each domain is divided into different specialized centers, with different key expertises. There are, in total, 44 key expertises (Figure 1).

1. Infectious Diseases and Vaccinology (Centre for Infectious Disease Control).
    - **a.** National Coordination Centre for Communicable Diseases Control.
    - **b.** Centre for Infectious Disease Epidemiology and Surveillance.
    - **c.** Centre for Research Infectious Diseases Diagnostics and Screening.
    - **d.** Centre for Zoonoses and Environmental Biology.
    - **e.** Centre for Immunology of Infectious Diseases and Vaccines.
2. Environment and Safety.
    - **a.** Centre for Safety of Substances and Products.
    - **b.** Centre for Sustainability, Environment and Health.
    - **c.** Centre for Environmental Quality.
    - **d.** Centre for Environmental Safety and Security.
3. Public Health and Health Services.
    - **a.** Centre for Health and Society.
    - **b.** Centre for Health Protection.
    - **c.** Centre for Nutrition, Prevention and Health Services.



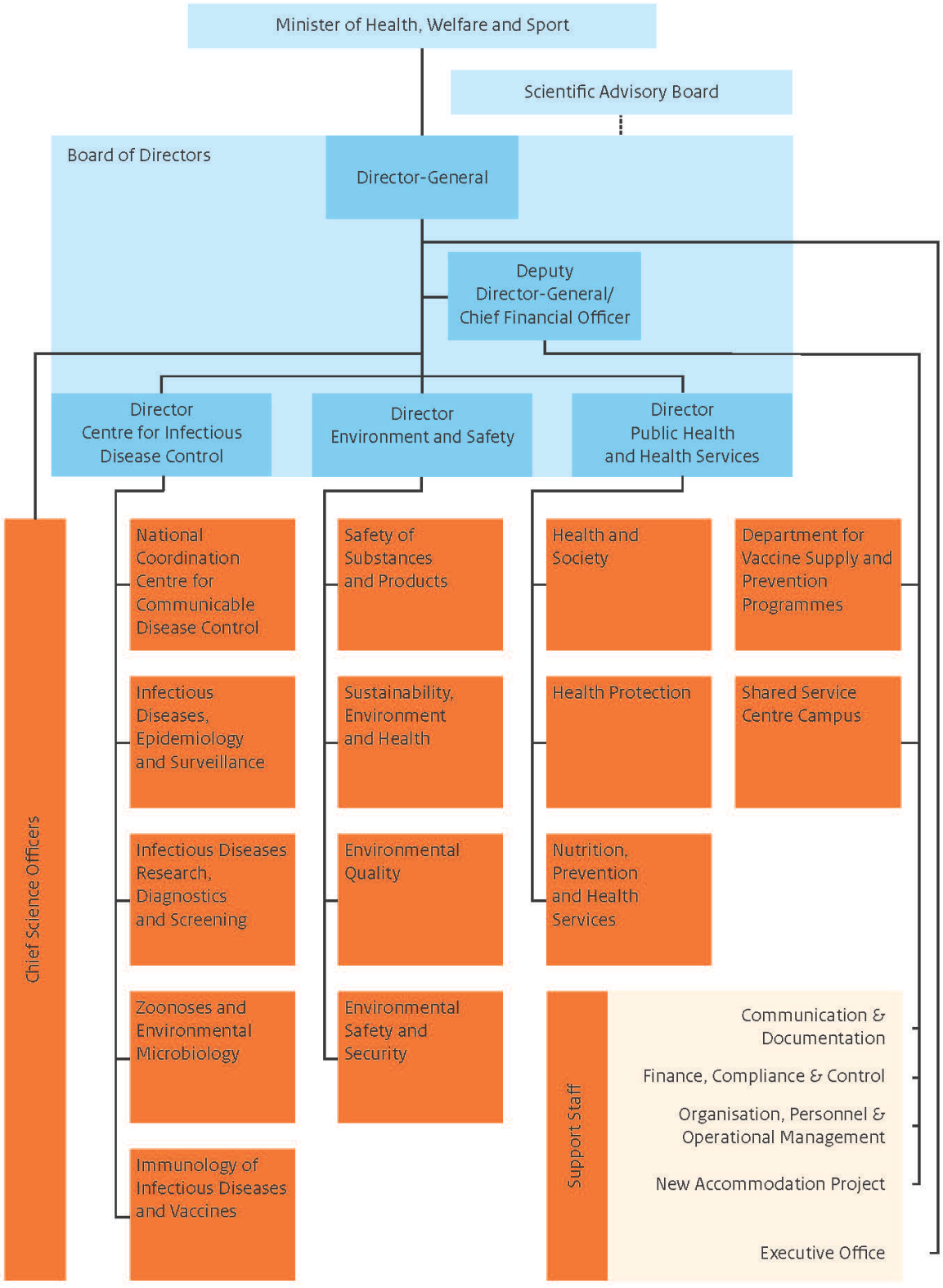
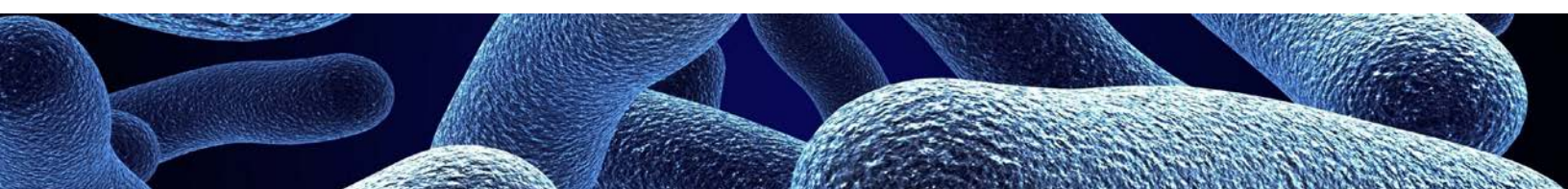


FIGURE 1: ORGANIZATION CHART OF THE RIVM [16].



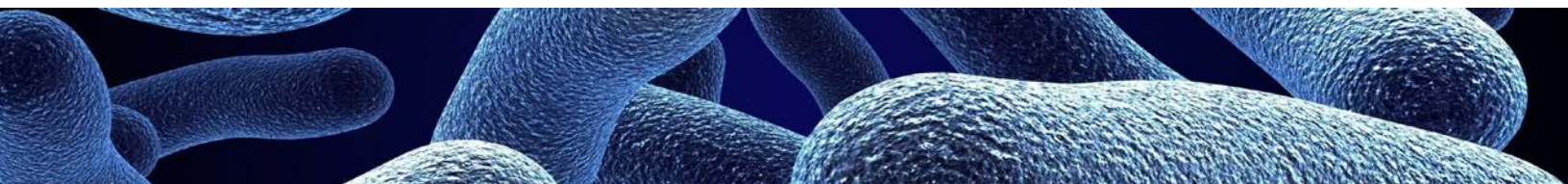
A director is the head of each domain, and together with the Director-General, the Deputy Director-General/Chief Financial Officer and the head of Executive Office, the Board of Directors is formed.

2.3. POSITIONING OF THE INTERNSHIP

The internship has been initiated by the Centre for Safety of Substances and Products (VSP), in cooperation with the Centre for Infectious Disease Control (CIb). VSP advises the government on the development and execution of policy and regulation regarding industrial chemicals, biocides, consumer safety, nanotechnology and genetic modification, among others. In addition, it develops new methods and guidelines for, for example, risk assessments, socio-economic analyses and risk reduction. Together with societal parties, such as producers, consumers, ngo's and citizens, VSP develops initiatives aimed at a responsible use of chemicals and products in a sustainable society. Synthetic biology is one of the key expertises of this centre [17]. VSP also has a signaling function when it comes to the developments that take place within this field. It provides its main client, the ministry of Infrastructure and the Environment (*Infrastructuur en Milieu; I&M)* with advice on these developments.

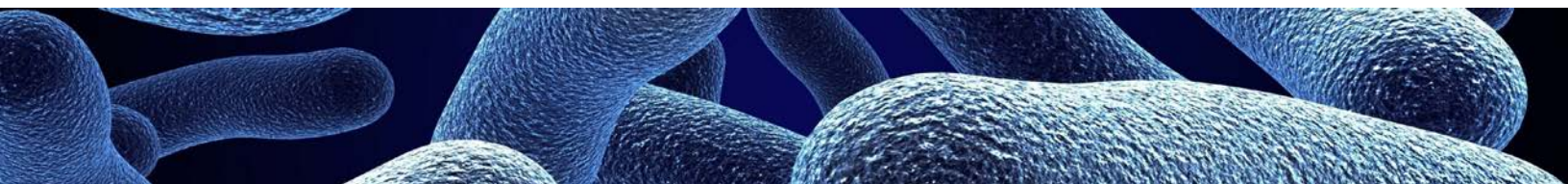
Antibiotic resistance on the other hand, is a key expertise of the CIb. The mission of the CIb is the signaling, control and prevention of infectious diseases for the benefit of human health in the Netherlands. It formulates the desired prevention- and control policy and advises the government and professionals. To this end, it performs its own scientific research. In the occurrence of a (national) outbreak, the CIb will coordinate and communicate its control. In addition, it contributes to the development of expertise, quality and uniformity in the control of infectious diseases [18]. In contrary to VSP, the CIb does not provide its client, the ministry of Health, Welfare and Sport (VWS), with knowledge on innovation in antibiotic development. This signaling function is executed by the Direction of Medicines and Medical Technology (*Directie Geneesmiddelen en Medische Technologie; GMT)* of VWS.

AMR is a global multifaceted health problem, for which many strategies are needed in order to tackle this problem. Developing new antibiotics and alternatives, as well as good diagnostics, is one of them. In the field of synthetic biology on the other hand, several developments are taking place, which could attribute to this strategy. This internship therefore provides a connection not only between two knowledge domains, but also between two centers.



Part 1

Developments in synthetic biology that can contribute to tackling AMR.



CHAPTER 3 – SYNTHETIC BIOLOGY

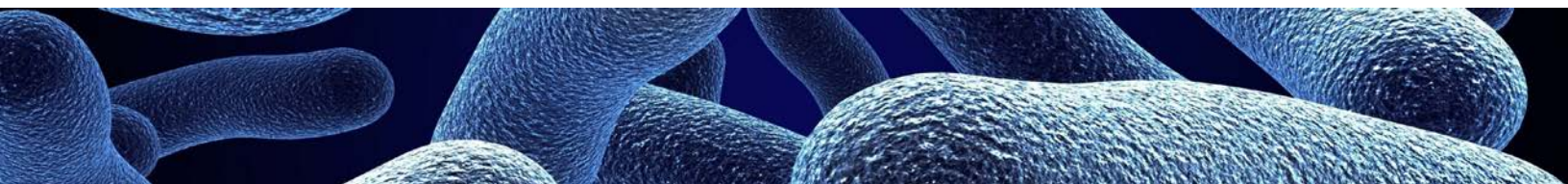
3.1. WHAT IS SYNTHETIC BIOLOGY?

Synthetic biology is an emerging research area, which aims at designing biological systems, components and processes, that do not already exist in nature, together with the re-design of existing biological systems [19]. Classical engineering concepts, such as standardization and modularization, are adopted and applied to biological systems [4]. Whereas in (molecular) biology it can be said that '*DNA makes RNA, and RNA makes proteins',* in synthetic biology this can be rephrased as '*parts make devices, and devices make systems'*[20]. It is an interdisciplinary field of research, including engineers, biologists, chemists, computer scientists and social scientists. In part due to this interdisciplinary nature, synthetic biology shows potential in the development of tools and applications in various parts of the industry, such as bioenergy, agriculture, medicine and biotechnology. In addition, although the primary aim of the field is the development of applications that lead to products, it can also aid in the understanding of various biological systems [21]. Its advances could provide the world with solutions to various global environmental and medical problems. It has the potential to create organisms that can produce clean fuels [22], to recognize and destroy tumors[23], or to modify plants in such a way that they can sense chemicals and respond to it[24, 25]. Another problem synthetic biology could provide answers to, is antimicrobial resistance (AMR), a (multidrug-) resistance of pathogenic bacteria towards antibiotics.

> *'SynBio is the application of science, technology and engineering to facilitate and accelerate the design, manufacture and/or modification of genetic materials in living organisms' (SCENIHR) [4-6]*

3.2. (NOVEL) BIOLOGICAL SYSTEMS AND NETWORKS

Nature itself has evolved many biological molecules that are logically connected into networks. Cells use these complex interconnected networks to dynamically control various biological processes, produce biological components and ensure survival. The field of systems biology aims to study these natural biological systems. A close relation between systems biology and synthetic biology therefore exists *(See textbox 'Synthetic biology vs systems biology')*. Synthetic biology however, aims to characterize, standardize, and expand the toolbox of biological building blocks. Using an engineering approach, these parts can be combined to create novel synthetic circuits, which can execute various functions after they are inserted into cells, or hosts. The circuits can be applied on transcriptional, translational or post-translational level, or on combinations thereof [26].



SYNTHETIC BIOLOGY VS SYSTEMS BIOLOGY

Synthetic biology complements systems biology. Whereas synthetic biology is based on forward engineering, building things that do not yet exist, systems biology is based on reverse engineering, having a better understanding of natural biology [9, 10]. The transformation in thinking in using forward design, in addition to the traditional reverse engineering approaches, has been said to explain the rapid growth of the field of synbio. Computational modelling tools have become essential in both fields. In systems biology, it aids the predictions about the behavior of a system, whereas it is used for direct design in synthetic biology [10].

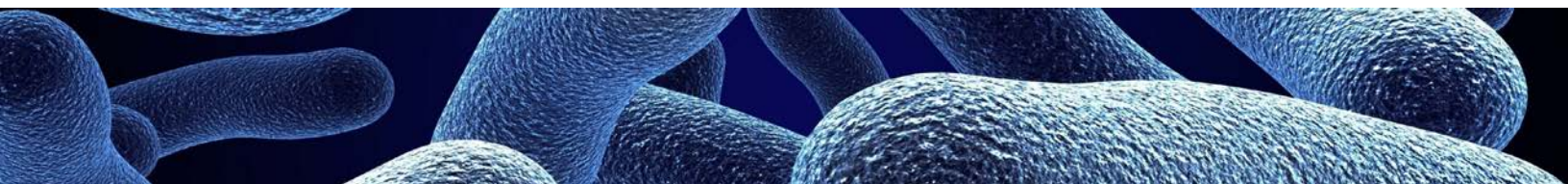
Two milestone papers, published in *Nature* in January 2000, stand at the base of this trans-disciplinary field. In these papers, the first synthetic gene networks were designed and constructed: an oscillator and a toggle switch [27, 28] . The biological equivalents of electronic memory storage and timekeeping, respectively, showed that engineering principles could be applied to biological systems [29]. Many other, more complex genetic switches [30, 31] and oscillators [32, 33] followed, as well as logic gates [34-36], memory elements [37, 38], timers [39], counters[40] and intercellular communication modules [41].

This so-called top-down approach aims to add parts, or genome sequences to an engineered organism, which acts as a chassis. In addition, another direction in the field of synthetic biology can be distinguished, namely the bottom-up approach. Here, the goal is to develop a basic form of life from scratch. It is not directly aimed at developing a technical application, but instead tries to understand the basic functions of life [42].

3.3. ITS ORIGINS

Over the last decade, synthetic biology has been recognized as a research area with the potential to significantly enhance our understanding of physiological health and can benefit the bioeconomy and society as a whole [19, 20, 43, 44]. Its origins can be traced back as far as the 1960s and is based on several areas of research [45]. In the 1970s, the development of the recombinant DNA technology, in which genes from one organism can be cloned and be inserted into another, had great impact on the field of molecular biology [46, 47]. The biotechnology industry flourished in the 1980s, exemplified by the large scale production of recombinant human insulin [48]. The following ‘omics era, included an understanding of transcriptional regulation (transcriptomics), the biochemical functions of all the gene products (proteomics), and the interactions between them (interactomics) [49]. *In silico* biology, using computational models, and systems biology, followed in the 2000s [50, 51].

Moreover, fast-paced development in electronics, improvements in sequence technology and the ability to synthesize DNA in a quick and cheap manner, have sparked the interest and the developments in synthetic biology in industry and academies [52].



3.4. THE IGEM COMPETITION

This increased interest is also exemplified by the enormous increase in competing teams in the iGEM competition, a student competition in synthetic biology (Figure 2). The iGEM competition, the Internationally Genetically Engineered Machine competition, started in 2003 as an independent study course at the Massachusetts Institute of Technology (MIT). It grew to be a summer competition with 5 teams in 2003, and with 13 teams in 2005. In 2015, it has expanded to 280 teams from more than 30 countries [53].

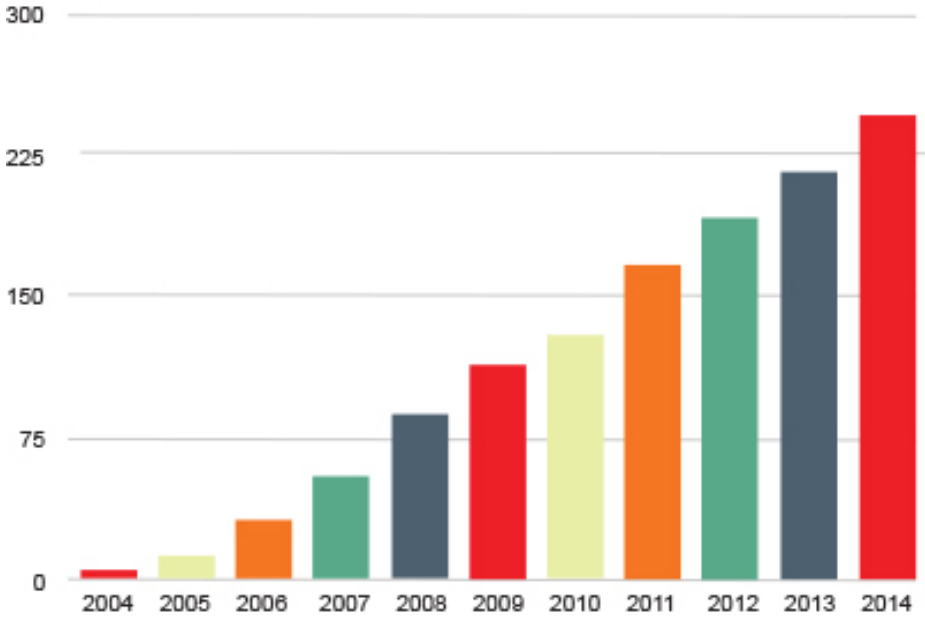
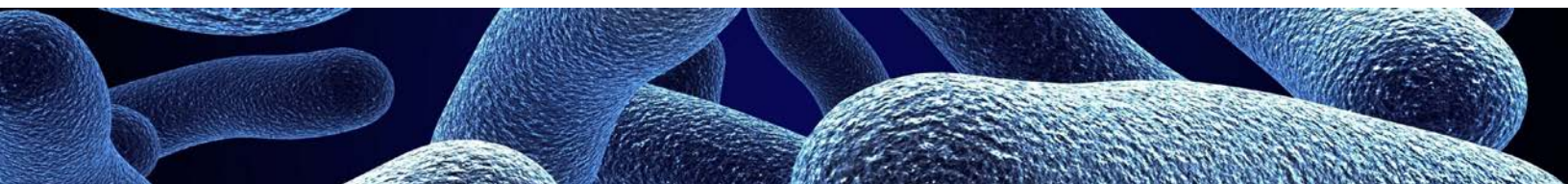


FIGURE 2: GROWTH OF TEAMS FROM 2004 TO 2014. ADAPTED FROM [54]

3.5. DEFINING SYNTHETIC BIOLOGY

One of the main challenges in explaining synthetic biology is defining what it encompasses. The many areas of research on which synthetic biology relies, makes a simple and uniform definition difficult. It is interdisciplinary and draws on precepts and and practices from chemical engineering, biochemistry, molecular biology, computer science, and many more[19]. In addition, a clear distinction between recombinant DNA technology in general, and synthetic biology is not always present. The novel element in synthetic biology is often one of scale. It is also not constrained in using genetic material that already exists in nature [19].

Although its definition may not always be uniform, it seems that its potential in contributing to societal needs and promoting economic growth is widely seen by various parties. The size of that potential however, is yet to be determined[4, 19].



CHAPTER 4 - ANTIMICROBIAL RESISTANCE

4.1. INTRODUCTION

Antimicrobial resistance (AMR), the resistance of microbes towards medicine, has been stated to be one of the most significant challenges to the health care sector in the 21st century and one of the biggest global health problems today. This has been acknowledged by many governments, organizations, scientists and institutions. The World Health Organization for example has identified AMR as one of the three biggest threats to human health [55].

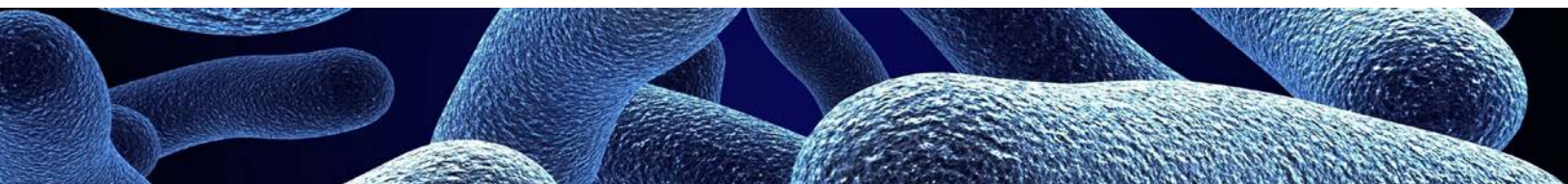
In the knowledge document in Appendix I.1. a more extensive description of antimicrobial resistance and its relevant aspects can be found. A short history on antibiotic development is given, as well a classification of the antibiotics we currently use. Their working mechanisms, and the bacteria that pose the biggest threat to our health will be highlighted as well.

Here, AMR will be introduced shortly, after which the difference between Gram-positive and Gram-negative bacteria will be explained. In general, the multi-resistant Gram-positive bacteria are the biggest health threat, as their extra outer membrane creates difficulties in targeting the bacteria. Lastly, a brief history on the methods used in antibiotic discovery will be given, and it will explain why this has failed to continue providing us with new antibiotics.

4.2 WHAT IS THE PROBLEM?

Antibiotics or antimicrobial agents control infection by bacteria and other microbes, and their use is a cornerstone in modern medicine [56]. However, bacteria and other pathogens have always resisted the drugs that are used against them. The systemic misuse and overuse of antibiotics has contributed to that greatly. In addition, the pipeline for antibiotics and alternatives is limited. Only 5 new antibiotics have been marketed since 2000, and none of them targeting the deadly and highly resistant Gram-negative bacteria [57]. A post-antibiotic era, in which common infections again can kill, is seen as a very real possibility [55]. The O'Neill Commission, commissioned by UK Prime Minister and the Wellcome Trust, estimates that, unless action is taken, an additional 10 million lives will be lost a year by the year 2050. An economic effect has been estimated as well, in which AMR will have a cumulative cost of 100 trillion USD [58]. Already, so-called 'superbugs' are being found. *E.coli* resistant to colistin, a last-resort antibiotic, was first found in pigs and in people in China in November 2015[59], and in May 2016 a woman in the US was infected with this bacterium[60].

AMR is a multi-faceted and complex problem, which poses scientific, economic, and policy challenges [56]. It requires both a focus on the supply of antimicrobials ('the pipeline') and the demand for them (consumption) [61]. In addition, antibiotic resistance is not restrained by country borders and interactions and connections between human, veterinary, wildlife and environmental health exist [62]. Our modern world, in which we created an elaborate infrastructure, only increases these connections.

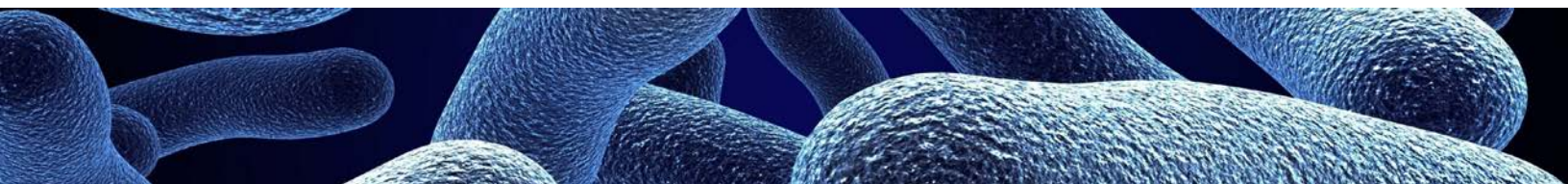


The Dutch government has recently proposed an action plan for the period 2015-2019, in which a 'One Health' approach towards combatting AMR is stated. It focuses on all areas in which the human health is threatened by multi-resistant bacteria: healthcare, animals, food and the environment. Part of this action plan was the EU presidency of the Netherlands in the first half year of 2016 [62].

The urgency of the problem is not only acknowledged by governments or institutions, but also by the industry. 85 companies, including large pharmaceutical, biotechnology and diagnostics companies, have stated in a declaration to be committed to further action to reduce drug resistance, increase research and improve access to antibiotics [63].

4.3. BACTERIA – POSITIVE VERSUS NEGATIVE

In general, bacteria can be divided into two classes, based on the Gram staining technique; Gram-negative and Gram-positive bacteria (Figure 3) [64]. Both types of bacteria have a cell wall, consisting out of layers of peptidoglycan. However, Gram- negative bacteria have an outer membrane covering a thin peptidoglycan layer, while Gram- positive bacteria lack this membrane and instead have a thick peptidoglycan layer. In the first step of the Gram procedure, a positively charged dye (crystal violet) is used, which binds to the negatively charged outer membrane of the Gram- negative bacteria. In the second staining step, the cells are washed with ethanol, which dissolves the outer membrane of the Gram-negative bacteria, thus de-staining them. The dye is however retained in the thick peptidoglycan layer of the Gram-positive bacteria, giving them a purple color. The pink or red color of the Gram-negative bacteria is caused by a counterstain [65, 66]. In general, Gram-negative bacteria are more resistant towards antibiotics, because of their additional outer membrane. Therefore, any antibiotic targeting these bacteria will have to be developed in such a way as to overcome this extra barrier.



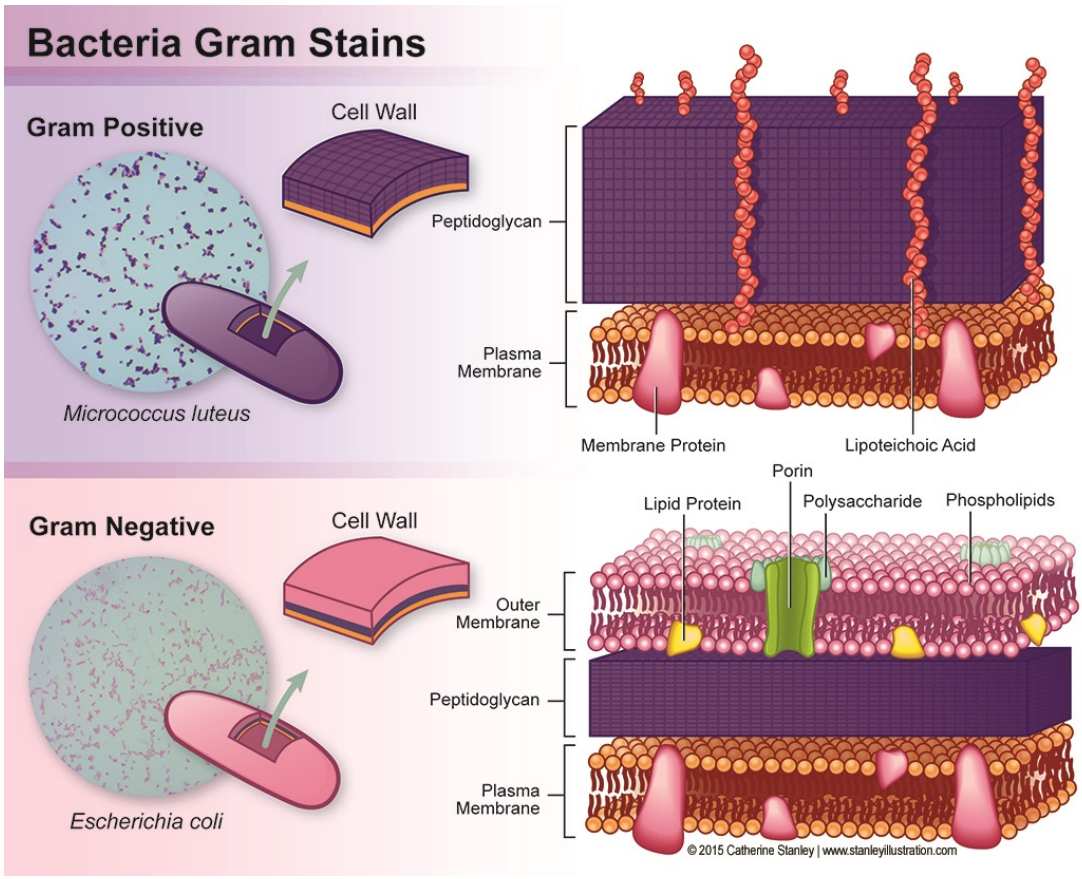
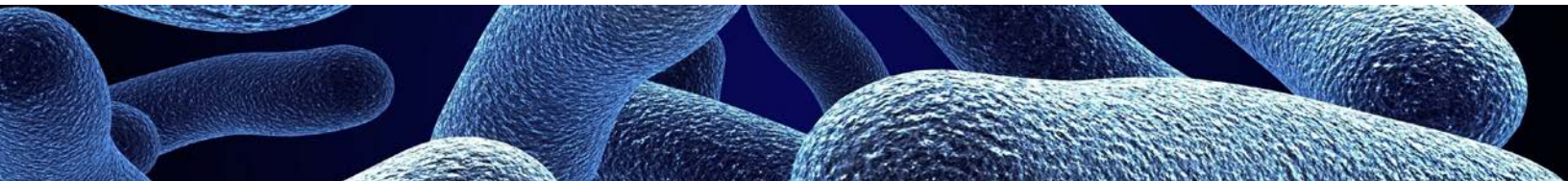


FIGURE 3: BACTERIA CAN BE CLASSIFIED BASED ON THEIR GRAM STAINING. ADAPTED FROM [67].

4.4. ANTIBIOTICS: AGENTS 'AGAINST LIFE'

The first antimicrobial agents were synthetic molecules, such as dyes, discovered by the screening of libraries of chemicals. However, the discovery of penicillin (1928)[68] and streptomycin (1943) [69], amongst others, caused the realization that bacteria and fungi in the environment could produce molecules that could treat bacterial infections in humans. Especially the Actinomycetes (the filamentous bacteria that also produce streptomycin) are exceptionally potent in the number of antibiotics that they produce, accounting for 75% of the antibiotics. About 75% of these are made by a single genus, the *Streptomyces* [70]. These discoveries led to the golden era of antibiotics, in which the products of the secondary metabolism of these bacteria and fungi were screened for antibiotic activity. In this period, most of the microbial natural antibiotics that we use today were discovered [71].



An unsuccessful approach

After the 1960's however, the 'low-hanging' fruit of the natural antibiotics was picked and the screening programs were mostly rediscovering existing antibiotics. In addition, another problem was arising: antibiotic resistance. It was for these reasons that the industry started to focus on the chemical modification of natural antibiotics with improved therapeutic properties, a field called medicinal chemistry, many of which proved to be very successful and still used today [72](Figure 4). During this synthetic tailoring, the core of the natural antibiotic, or scaffold, is used and chemical groups at its periphery are modified to improve its properties [73]. Yet despite the success of these natural products, the pharmaceutical industry turned away from these molecules in the last two decades, in favor of target- based drug discovery. In this approach, proteins are selected and characterized *in vitro*. These 'validated targets' are then screened in large chemical libraries, a process called high-throughput screening (HTS). The affinity and potency of the 'hit' towards the target is then characterized [74]. This approach however, has not led to any new antibiotics brought to the market in the last 20 years [75]. The synthetic molecules in those libraries are not tailored for microbial biology and are mostly focused on their bioactivity in humans [76]. Meanwhile, multidrug resistance is emerging, causing a global necessity for new antibiotics [77].

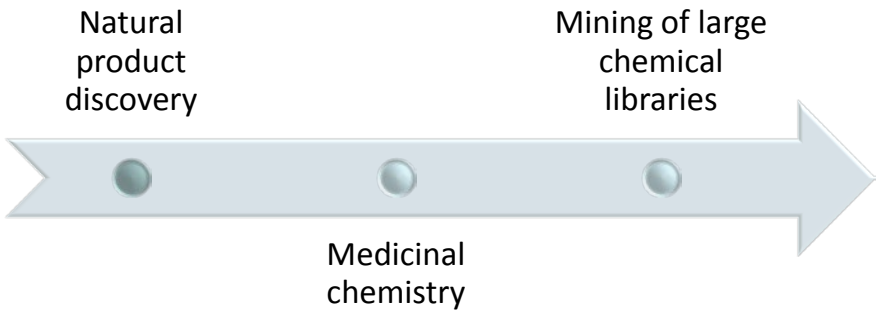
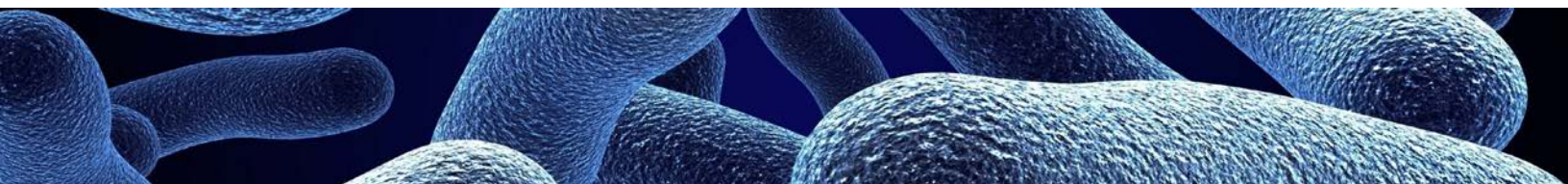


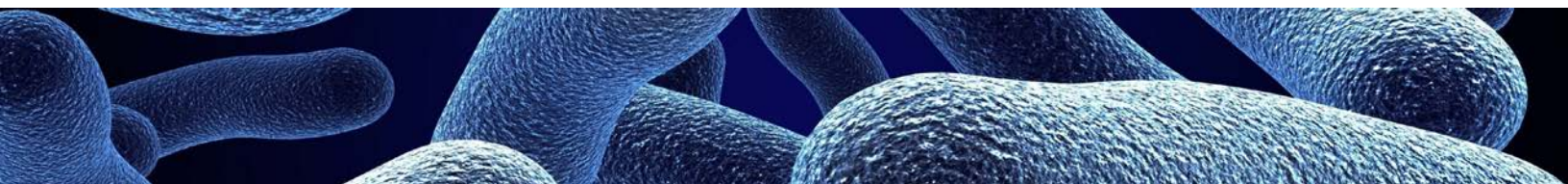
FIGURE 4: MOST ANTIBIOTICS ARE EITHER 1) NATURAL PRODUCTS FROM MICROORGANISMS 2) ARE SEMI-SYNTHETICALLY PRODUCED USING MEDICAL CHEMISTRY 3) OR ARE CHEMICALLY SYNTHESIZED [78].



CHAPTER 5 - THE POSSIBILITIES OF SYNBIO IN AMR

The need for new strategies to combat pathogens, especially those resistant to multiple drugs, is increasing. The field of synthetic biology offers new opportunities to solve this resistance problem. This includes the *discovery of new antibiotics* (either natural, or new-to-nature) and the use of *alternatives to antibiotics* such as bacteriophages. In addition, *new diagnostics* are being developed to enhance the detection of a specific pathogen (Figure 5). In this chapter, the main concepts underlying these developments will be given, occasionally illustrating these using examples. These examples have been chosen for their illustrative purposes, and no ranking on importance has been made. For a more extensive and detailed overview of other examples within these developments, the following chapter can be used together with the knowledge document in **Appendix I.**

FIGURE 5: STRATEGIES INVOLVING SYNTHETIC BIOLOGY IN TACKLING AMR. *BLUE: NOVEL ANTIBIOTICS. ORANGE: ALTERNATIVES TO ANTIBIOTICS. GREEN: DIAGNOSTICS.*



5.1. DISCOVERY OF NEW ANTIBIOTICS

To identify new antibiotic candidates using synthetic biology, several approaches are being exploited. This includes the identification of new drug targets, the development of screening platforms for the identification of new drug candidates, the discovery of novel natural products, or creating new-to-nature molecules. In figure 6 these developments are summarized and a short description is given. In the following chapter, each development will be explained in more detail.

Novel antibiotics (natural or new-to-nature)

- Removal of all native regulation and non-essential genes. Natural genetics are rewritten, in order to produce compounds that are normally not produced by the organism. This can be achieved via the awakening of 'silent' gene clusters, or the creation of new gene clusters for new-to-naure molecules.
- *Examples: novel-small molecule antibiotics, novel peptide antibiotics, non-traditional antimicrobials: bacteriocins (See appendix I.2.)*

Minimal genome approach for target identification

- Identification of novel drug targets either via removal of all non-essential genes (top-down approach), or by synthesizing a genome with only essential genes (bottum-up approach).

New antibiotic screening platforms

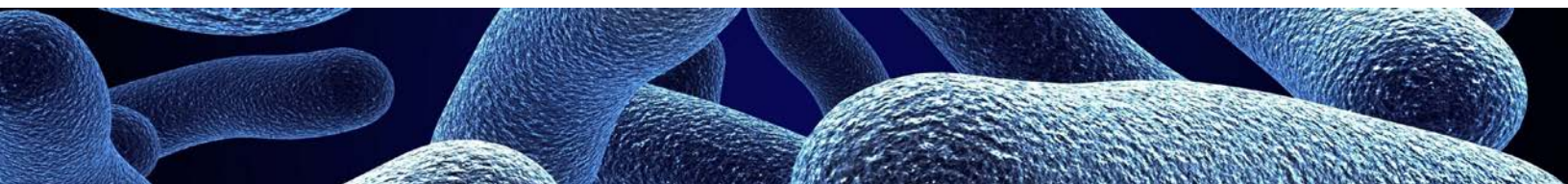
- Engineering of a synthetic gene network that can sense molecules and provide an output, in order to screen for new antibiotics.

FIGURE 6: SYNBIO DEVELOPMENTS THAT CAN LEAD TO (THE DISCOVERY OF) NOVEL ANTIBIOTICS.

5.1.1. THE UNSUCCESSFUL SEARCH FOR NEW NATURAL ANTIBIOTICS

It has been proven difficult to find new natural antibiotics. For the last two decades, the discovery of new classes of antibiotics, with novel mechanisms of actions, has been scarce. A rediscovery of known molecules has taken place instead. Fortunately, we have increased our knowledge of the production pathways of these molecules. More importantly, new technologies have been developed, such as bioinformatics and synthetic biology. Together, these developments can provide us with new opportunities to create new natural antibiotics.

An important characteristic of natural antibiotic products is their complexity and diversity, due to their interaction with proteins, nucleic acids and membranes under physiological conditions, and their selection over millions of years of evolution [71, 75]. Therefore, the value of natural antibiotics is greatly appreciated. However, together with their current low discovery rate, it is difficult to predict the drug qualities of the natural compounds that are found, as their chemical structures are complex [79].



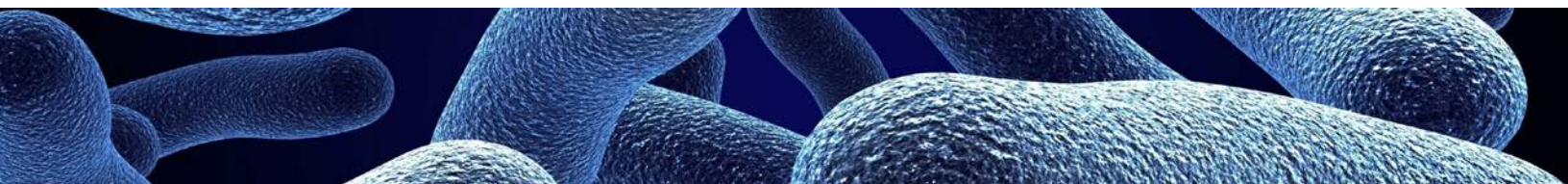
On the other hand, the focus on synthetic molecules has also not been fruitful. With the exception of the synthetic antibiotic linezolid, three of the four new classes of antibiotics are derived from natural products: the lipopetide daptomycin, the diterpene retapamulin and the macrocyclic polyketide fidaxomicin. This innovation gap has been somewhat obscured by the development of semi-synthetic derivatives from natural products, or molecules inspired by well-known natural products upon revisiting old scaffolds [80]. Synthetic biology could offer us the possibility to overcome this innovation gap, by aiding in the discovery of new natural antibiotics. In addition, it offer the possibility to create novel molecules that do not already exist in nature, the so-called new-to-nature molecules.

5.1.2. HOW CAN SYNTHETIC BIOLOGY HELP?

Synthetic biology can offer solutions to sample the large chemical space of natural products and its derivatives [81]. Nature has a large pool of bioactive secondary metabolites, which can function as an antibiotic. However, its discovery is slow, urging the need for new strategies to fully exploit this large chemical space. These strategies aim at the construction of new biosynthetic gene clusters or the activation of so-called ‘silent’ or ‘cryptic’ gene clusters in optimized (heterologous) hosts.

Biosynthetic gene clusters (BCGs)

In the genomes of the microbes that produce these natural products, the genes that encode for this production are generally clustered together in so-called biosynthetic gene clusters (BGCs) [75]. So far, the experience is that these clusters consist of core genes that are required to build the chemical scaffold, as well as the genes that encode for the enzymes that tailor these scaffolds. In addition, it holds the genes that are necessary for compound efflux, gene regulation and self-resistance [76]. An example of a BCG, namely the BCG of the antibiotic daptomycin, is shown in Figure 7. This co-localization of the genes that are involved in the production of secondary metabolites facilitates the characterization of biosynthetic pathways, such as the assembly of polyketide antibiotics, or non-ribosomal peptide antibiotics [82]. Researchers used to rely on the creation of random mutations, deletions, replacements or introductions into the genomes of the antibiotic-producing bacteria for the production of new compounds, an approach called combinatorial biosynthesis [83]. This process was later refined, and it was shown that libraries of novel compounds could be produced applying systematic and rational mutations of an antibiotic biosynthetic pathway. In addition, this machinery could be placed in a heterologous host, such as E.coli [84]. Over the years, the field has evolved itself in order to make itself applicable and compatible with modern drug discovery, aiming at an increase in the diversity of natural antibiotic products [75].



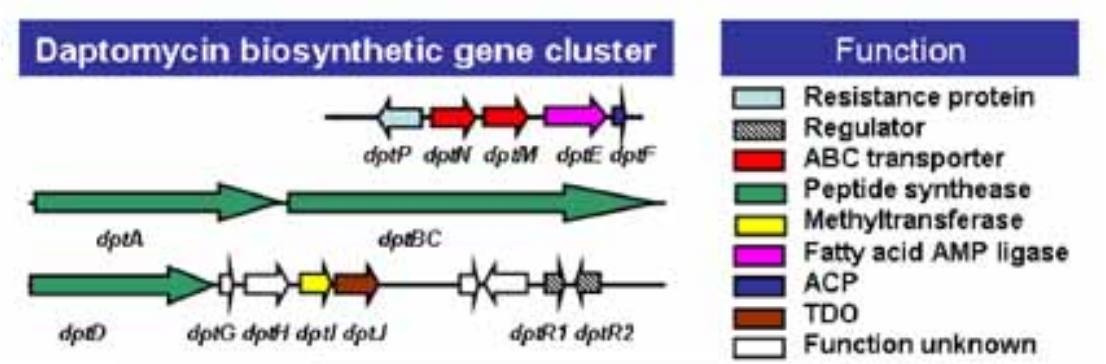


FIGURE 7: BIOSYNTHETIC GENE CLUSTER OF THE ANTIBIOTIC DAPTOMYCIN. ADAPTED FROM [82].

Rewriting natural genetics

In order to increase the diversity of natural antibiotic products, the natural genetics of the microbial producer are 'rewritten'. In the so-called refactoring process, all native regulation, such as promotors and transcription factors, as well as non-essential genes, are removed [83, 84]. The BGCs of natural products are large and complex, and have elaborate native regulation. This makes it difficult to change the expression levels of individual genes in a BGC [85]. Thus, by refactoring a gene cluster, and introducing new, controllable regulation, the desired end compound can be produced.

This way, new gene clusters can produced and so-called 'silent', 'sleeping' or 'cryptic' gene clusters can be 'awakened' [86] (Figure 8). Previous attempts to 'awaken' these gene clusters, by forcing these cells to express the genes in those clusters, have been successful [87-89]. However, high-throughput screening, for quick characterization of large amounts of clusters, was not yet developed. More importantly, these clusters often originate from various organisms, which are difficult or impossible to culture [86]. Thus although large numbers of gene clusters have been identified, many of their natural products are not produced at detectable levels under laboratory conditions.

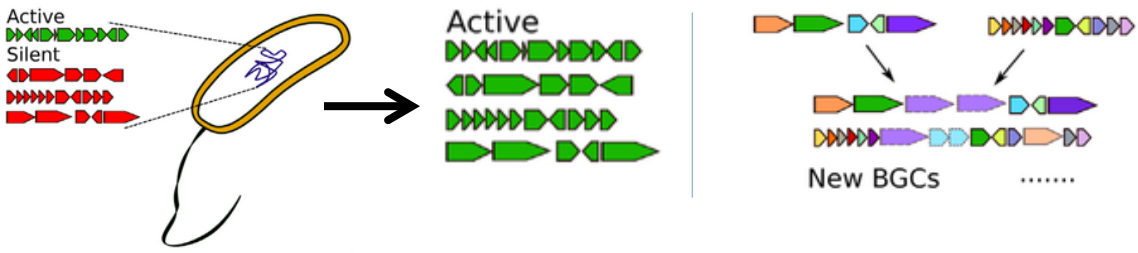
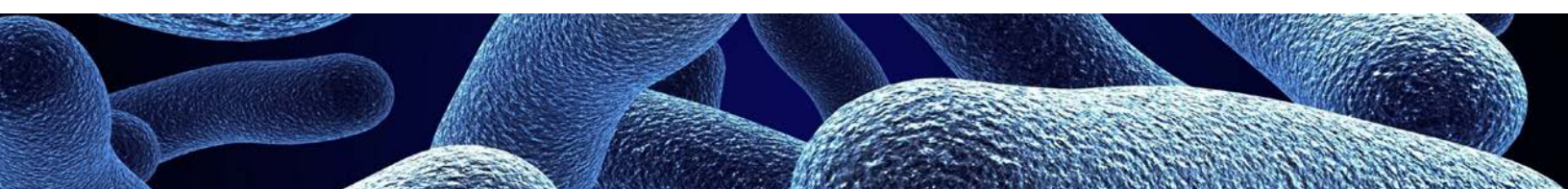


FIGURE 8: SYNTHETIC BIOLOGY CAN AID IN THE ACTIVATION OF 'SILENT' GENE CLUSTERS, OR THE CREATION OF NEW BCGS. ADAPTED FROM [2].



How does it work?

In figure 9, a model of the application of synthetic biology to expand antibiotic diversity is shown [75]. First, via computational selection, the gene clusters that have the highest potential to produce novel drug leads can be identified. This process is aided enormously by the large amount of DNA sequence information that is already present in large strain banks and sequence databases [90]. Bioinformatics can predict the gene clusters that encode for the machinery to produce these molecules. DNA synthesis can convert this information back to physical DNA [10]. Thus, instead of screening millions of microbes, the BCGs producing antibiotics can now be found by scanning genomes[79]. For example, Medema et al. developed a plug-and-play strategy in which algorithms were created to identify these gene clusters [91][Appendix II.7]. This also leads to the identification of the so-called parts, consisting out of the genes encoding for various chemical scaffolds (such as polyketides or peptides) and tailoring enzymes (such as isomerases or acyltransferases).

The parts are assembled into devices, gene assemblies encoding for specific compounds. These circuits can be new, as well as existing. In addition, the biological systems creating these natural antibiotics can be designed in a highly directed fashion, using a series of predictable and unifying rules of assembly and production[75]. Next, the devices are co-expressed in a suitable host, the chassis, which can generate output, the novel compounds. These systems can be created in the natural host, in other producers of similar scaffolds, or moved into an alternate host optimized to produce the product in high yields [76]. Finally, the produced products have to be screened via high-throughput screening or *in vivo* biosensors using light or fluorescence- based output. This way, the chemical diversity of antibiotics can be expanded using directed engineering of the gene clusters that produce natural products [79].

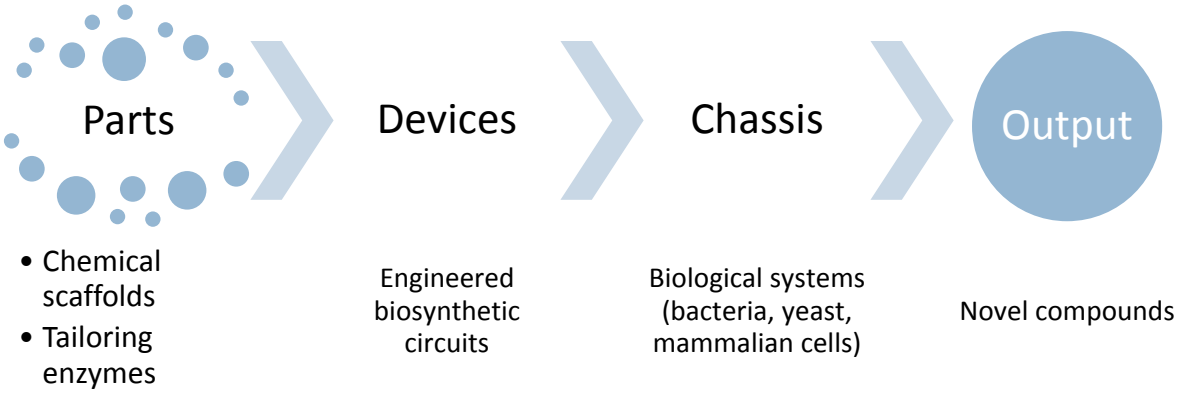
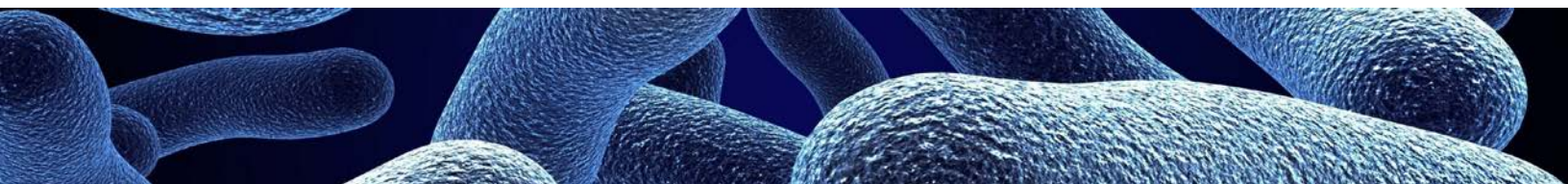


FIGURE 9: A MODEL OF THE APPLICATION OF SYNTHETIC BIOLOGY TO EXPAND NATURAL PRODUCT ANTIBIOTIC DIVERSITY. ADAPTED FROM [75]

In the previous chapters, a general approach in using synthetic biology in the production of novel antimicrobials has been described. In **Appendix I.2.** several ways such an approach is used will be explained in more detail: the production of *novel small-molecule antibiotics*, *novel antimicrobial peptides*, or *non-traditional therapeutics* such as bacteriocins [92].



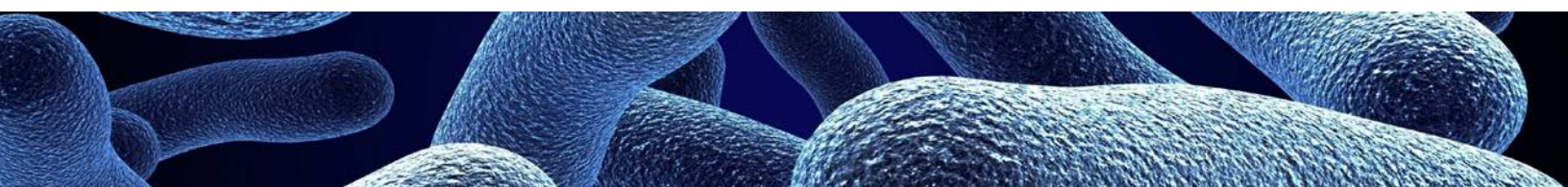
5.1.3. THE MINIMAL GENOME APPROACH FOR TARGET IDENTIFICATION

One of the ways synthetic biology influences the discovery of new antibiotics, is via the identification of new drug targets. Theoretically, the screening of the bacterial sequence database could provide us with a list of essential genes for compound screening. However, an efficient approach in doing so does not yet exist [93]. This difficulty could be overcome using synthetic biology, via the research on so-called minimal genomes. This provides researchers with knowledge of the essential genes of a cell. A top-down approach, by removing all non-essential genes, or a bottom-up approach, synthesizing the genome containing all of its essential genes, is used. Studying these genomes, especially those in antibiotic resistant bacteria, can provide us with novel drug targets.

High-throughput assays were developed to find compounds that would block the function of essential gene products or that would bind to essential proteins of unknown function from *Streptococcus pneumoniae* and *Staphylococcus aureus [94].* In the high-throughput screening (HTS) process, a library of compounds is used to quickly perform millions of tests, identifying an active compound that targets the product of interest. This way a library of compounds was screened for an active compound targeting the essential gene products or essential proteins of *S. pneumoniae* and *S.aureus*. Unfortunately, it failed to find new antimicrobials with clinical potential, an outcome that could be mainly attributed to the lack of chemical diversity in the compound libraries [74]. However, some antimicrobial candidates have been found for several other bacteria, including *Helicobacter pylori* and *P.aeruginosa [95, 96].* Although more work is needed, it does demonstrate the attractiveness of the study of these essential genomes

5.1.4. NEW SCREENING PLATFORMS

Synthetic circuitry can also be designed to screen for novel antibiotics. Usually, to detect antibiotics, candidate antimicrobial producers had to be isolated and characterized. In addition, test batches for several screening assays had to be produced [7]. This process is time-consuming and costly, a major reason why large pharmaceutical companies have focused on rational drug design [97].
To overcome these issues, synthetic biology has also focused on the development of other screening methods. A synthetic gene network can be engineered to sense (small) bioactive metabolites. Subsequently, a fluorescent reporter gene is activated, which provides an output. Libraries of candidate compounds can be screened by adding them to these cells, and observing the gene expression readout. Here, two examples of screening platforms are given. The first example describes a synbio approach towards the identification of new streptogramin antibiotics. The second example describes the identification of anti-tuberculosis drugs using synthetic gene circuits.



EXAMPLE 1: IDENTIFICATION OF NEW STREPTOGRAMIN ANTIBIOTICS

An early example of the use of synthetic circuitry as a screening platform, is the design of a streptogramin-controlled transcriptional switch by Aubel et al [7]. This platform allows libraries of metabolic compounds to be added to mammalian cells, after which they can be screened for the production of streptogramin antibiotics. The switch uses a direct molecular interaction between streptogramin antibiotics and a bacterial sensor. After addition of a streptogramin-containing culture or a single microbial colony, the antibiotic-protein interaction takes place. This signal is linked to an engineered transcription set-up and leads to modulation of the reporter gene expression. Although this system is used for the detection of streptogramin production, the same technology can be applied to screen other classes of antibiotics.

EXAMPLE 2: IDENTIFICATION OF ANTI-TUBERCULOSIS DRUGS

Another example is provided by the identification of anti-tuberculosis drugs using synthetic gene circuits [3]. Ethionamide is a last-line drug against tuberculosis, caused by *Mycobacterium tuberculosis.* In the bacterium, the pro-drug ethionamide is converted by the ethionamide activator (EthA) into a pathogen-killing metabolite, which is effective against *M.tuberculosis*. In the presence of the corresponding antibiotics, the ethionamide repressor (EthR) binds to a specific operator (O_{ethR}) and inhibits EthA, causing resistance of the bacterium against ethionamide. Therefore, compounds that would inactivate EthR, could trigger the activation of EthA, and increase the sensitivity of *M. tuberculosis* to ethionamide. Weber et al. [3] designed a synthetic gene circuit that senses the interaction between EthR and O_{ethR} and produces a quantitative reporter gene expression readout (Figure 10). This way, a novel drug candidate was identified. In principle, this screening method could be extended to other bacteria using pathogenic resistance mechanisms relying on DNA-binding proteins.

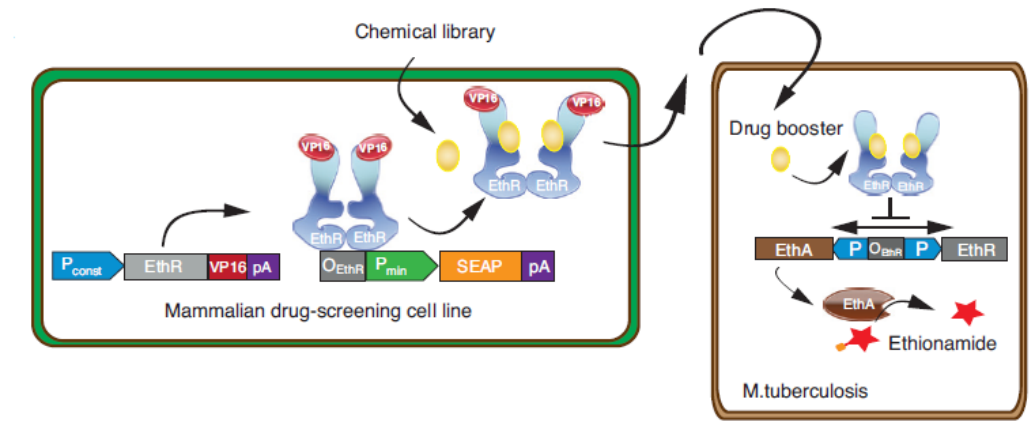


FIGURE 10: SYNTHETIC SCREENING SYSTEM FOR DRUGS THAT SENSITIZE THE PATHOGEN AGAINST ETHIONAMIDE. ADAPTED FROM [15].

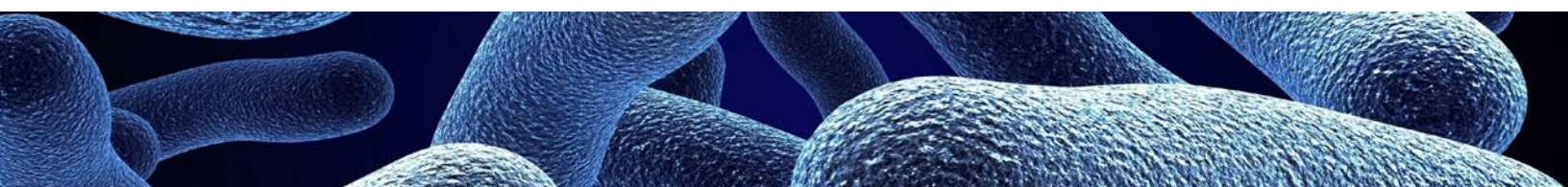
5.2. ALTERNATIVES TO ANTIBIOTICS

Microbes will always evolve in order to become resistant against the antibiotics that we use. In addition, because there is no guarantee that we will be able to find enough antibiotics to tackle AMR in the long-term, we should widen our focus [98]. Alternative approaches, defined as those that are different from our current antibiotics, therefore have to be considered as well. It should be noted that within this definition, bacteriocins (explained in Appendix I.2) could be defined as an alternative as well. However, for a better explanation of the synthetic biology approach in developing new molecules, it is placed in the previous chapter.

The importance of the development of alternatives to antibiotics, is also highlighted in a report, written by 24 scientists from academia and industry. The report was commissioned by the Wellcome Trust, and jointly funded by the Department of Health (England) and it assessed whether alternatives to antibiotics could contribute to controlling the rise of multi-drug resistant infections [99]. It states that the quest for successful alternatives is primarily limited by a lack of funding, and not a lack of options. After assessing 19 current alternatives to antibiotics, they have concluded that the first wave of alternatives will probably serve as an adjunctive or preventive therapy, and traditional antibiotics are still needed. The report estimates that £1.5 billion over the next 10 years is needed to develop the key alternatives and to advance them to the clinical stage. The most advanced novel therapies identified are probiotic-based treatments for *Clostridium difficile,* a common cause of diarrhea. Other alternatives, although progressing more slowly, include vaccines, antibodies, bacteriophages and the enzymes they produce, called lysins. It was estimated that new products could be expected as early as 2019 for *C.difficile* (antibodies, vaccines or probiotics), 2021 for *P. aeruginosa* (antibodies or vaccines*)* and 2022 for *S. Aureus* (antibodies, lysins or vaccines). Concern was raised over the little activity in the development of alternatives to the other ESKAPE pathogens or on the other Enterobacteriae. The report stated that it was unlikely that alternatives to antibiotics for these life-threatening pathogens will be developed over the next 10 years [99].

The role of synthetic biology

Synthetic biology can play a role in a variety of these alternatives to antibiotics, for example by enhancing their functionalities. These alternatives can either be preventative, such as vaccines, or therapeutic. In addition, they can be used instead of antibiotics, or as an adjunctive, accompanying antibiotic use. In **figure 11,** an overview of the alternatives to antibiotics in which synthetic biology could play a role are given.



Probiotics: 'sense and destroy'

- Non-pathogenic bacteria can be engineered to first sense the specific pathogen, and subsequently destroy it or inhibit its functions using various mechanisms.

Engineered bacteriophages

- Bacteriophages, viruses that target bacteria, can be engineered to carry out various functions. This include destroying the bacterial biofilm or sensitizing the pathogens for antibiotics, for example by removing the antibiotic resistance genes.

Engineered lysins

- Endolysins, enzymes produced by bacteriophages to digest the cell wall of the infected bacterium, can be engineered in order to enhance their functionalities.

Vaccine development

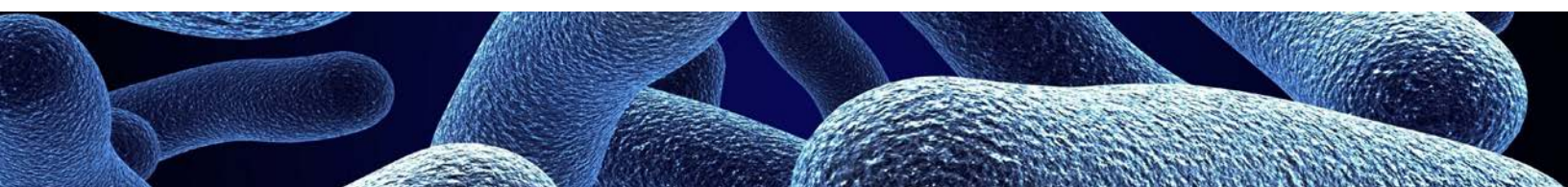
- It has been proposed that so-called 'smart vaccines', based on RNA circuits, would be a revolutionary alternative to our current vaccines. These vaccines provide an immunologist the control over the production of antigens and adjuvants in a desirable manner by triggering the response with small-molecule drugs.

FIGURE 11: THE INFLUENCE OF SYNTHETIC BIOLOGY ON ALTERNATIVES TO ANTIBIOTICS.

5.2.1. PROBIOTICS: 'SENSE AND DESTROY'

The benefit of using broad-spectrum antibiotics relies on their potential to be effective against a broad range of unidentified (pathogenic) bacteria. However, this also causes an inhibition of the growth of non-pathogenic bacteria of the human microbiome. A healthy, normal microbiome can compete with these with these pathogenic bacteria, making an individual less susceptible for the infections they cause [100]. Narrow-spectrum antibiotics on the other hand, leave the microbiome mostly intact by targeting a selected group of bacterial types.

Using synthetic biology, bacteria can be targeted more specifically, as is achieved with narrow-spectrum antibiotics. This method relies on a so-called 'sense and destroy' mechanism. Non-pathogenic bacteria can be engineered to first sense the specific pathogen, and subsequently destroy it or inhibit its pathogenic function using various mechanisms. To illustrate this, the targeting of the pathogenic *Pseudomonas aeruginosa* using this approach will be explained here. In Appendix I.3., two other approaches are mentioned: using engineered *E.coli* as probiotics to target *Vibrio cholerae*, and using engineered *L.lactis* in a bandage to prevent wound infection with *P.aeruginosa* or *S.aureus* (an example from the iGEM competition).



Targeting Pseudomonas aeruginosa

Two research groups used a synbio approach to target the pathogenic *P. aeruginosa* bacteria, a major cause of urinary tract and nosocomial infections. Saeidi et al. [101] and subsequently Gupta et al. [102] engineered non-pathogenic *E.coli* bacteria to express bacteriocins, antibiotic peptides produced by bacteria, upon sensing *P. aeruginosa.* To this end, they exploited the production of the bacteriocin pyocin by *P. aeruginosa,* by inserting these production genes into *E.coli*. Pyocins are normally produced by *P. aeruginosa* in order to compete with its own species in a competitive environment.

In the presence of the pathogenic bacteria, the *E.coli* produced the pyocins. Detection of the pathogenic bacteria took place by exploiting their communication systems. Whereas humans communicate via speech, bacteria use diffusible molecules as a chemical language, a process called quorum sensing. This way, they can program and coordinate behavior, causing population-wide behavior[15].

The subsequent release of pyocin was achieved either via the production of a lysis protein, specific for the membrane of *E.coli [101],* or a bacterial flagellum [102]. In the latter case, the self-lysis of *E.coli* is avoided, as the membrane remains intact. This approach can therefore support the release of different toxins, or different killing strategies, if the bacterium becomes resistant towards this specific bacteriocin (Figure 12).

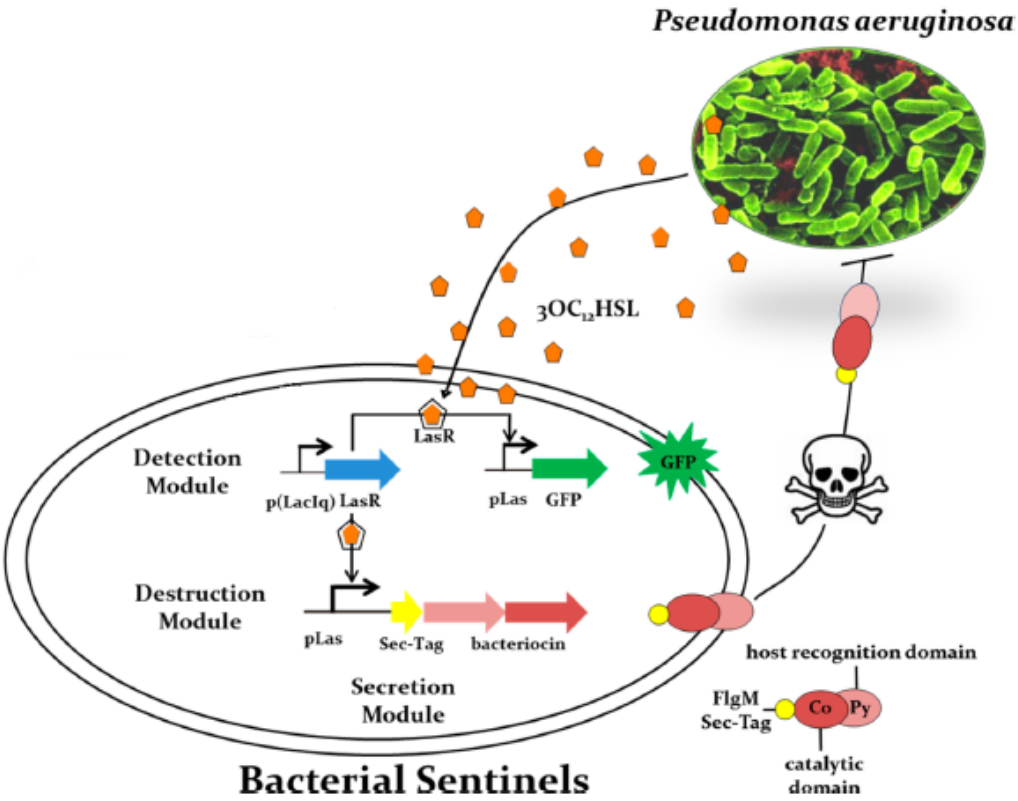
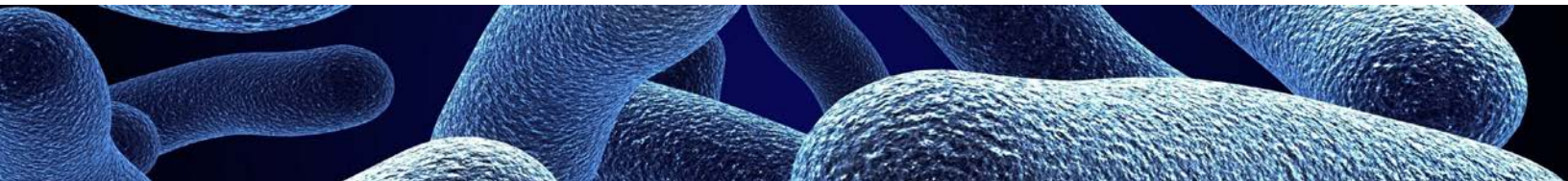


FIGURE 12: 'SENSE AND DESTROY' MECHANISM. THREE DIFFERENT MODULES ARE USED: A DETECTION MODULE, A DESTRUCTION MODULE AND A SECRETION MODULE. ADAPTED FROM [102].

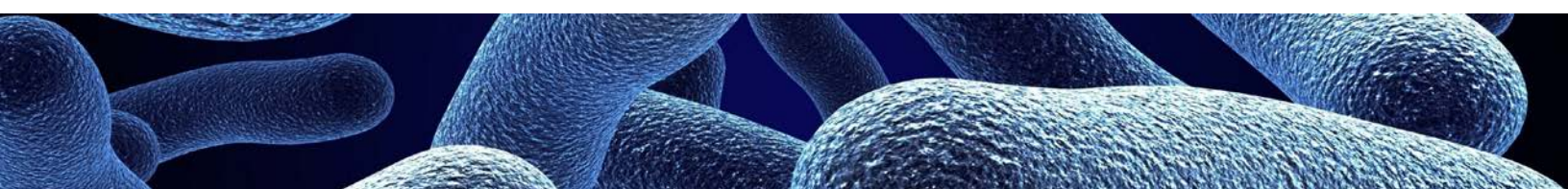


Limitations and solutions

A notable limitation in both these studies however, is their dependence on the diffusion of the bacteriocins towards the target bacteria. In addition, most bacteria do not live in a pure culture of cells, but in mixed communities called a biofilm. Biofilms are structured aggregations of surface-associated bacteria. These bacteria are encased in a self-produced matrix consisting of extracellular polymeric substance (EPS), which shields the bacteria from antibiotics or the host immune system. Biofilms play a critical role in the pathogenicity of bacteria and the process of infection [103]. Indeed, reports show that about 80% of microbial infections involve the formation of a biofilm [104].

In order to overcome these problems, Hwang et al. engineered *E.coli* to specifically recognize, migrate toward, and eradicate both dispersed and biofilm-encapsulated *P.aeruginosa* [105]. After detection of the pathogen via their quorum sensing molecules, two molecules were expressed: the antimicrobial peptide microsin S for cell killing, and a nuclease for the degradation of the biofilm. The *E.coli* bacteria then 'swam' towards *P.aeruginosa,* via the sensing of the released quorum sensing signals. This resulted in refined antimicrobial and anti-biofilm activities.

Although promising, the efficacy of these methods remains to be shown *in vivo.* In addition, the use of live *E.coli* in the respiratory tract poses several challenges. The introduction of live bacteria into a patient requires intensive clinical testing and the bacteria might have to be able to self-destruct [106]. However, it might be beneficial for treating infections in the gut, where bacteria are already abundant and exist symbiotically. Engineered bacteria could therefore reside well in this environment [107].



5.2.2. ENGINEERED BACTERIOPHAGES

In the early 20th century, two microbiologists, Felix d'Herelle [108] and Frederick Twort [109] , independently from each other discovered bacteriophages. A bacteriophage, or phage, is a virus that infects a bacterium and uses the host machinery to replicate itself. Their name stems from their ability to subsequently lyse the bacterial host cells (Greek: to devour bacteria), releasing virion progeny that can continue the cycle, even in other sites of infection in the body [110]. Although enthusiasm rose, and bacteriophages were successfully used for several decades, the use of phage therapy declined in the Western world. This was caused not only by a poor understanding of its basic biology and conflicting therapeutic results, but also by the emergence of antibiotics [111, 112]. Phages did however remain to be an important tool in the study of genetics, molecular biology and bacteria [113].

Around 5500 different bacteriophages have been discovered so far, each of which able to infect one or sometimes several types of bacteria [114]. Phages are able to infect host pathogens via two pathways, the lytic and the lysogenic cycle. Lytic phages, undergoing the lytic cycle, kill their host directly after the production of new virion particles. Temperate phages are able to undergo both cycles. In the lysogenic cycle, they remain dormant, often by integrating their viral genome into the host chromosome. This cycle can therefore be used to transfer desired DNA into the bacteria [115] (Figure 13). Phages have several advantages and disadvantages. These have been summarized in Appendix I.4.

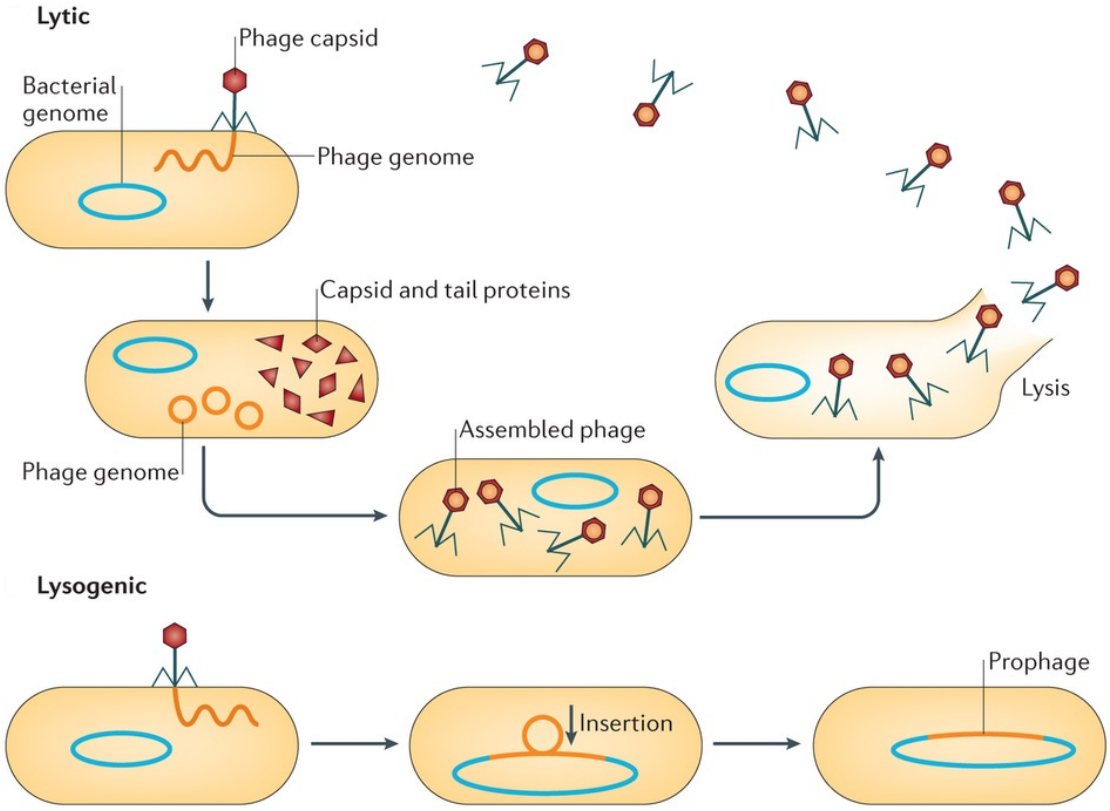
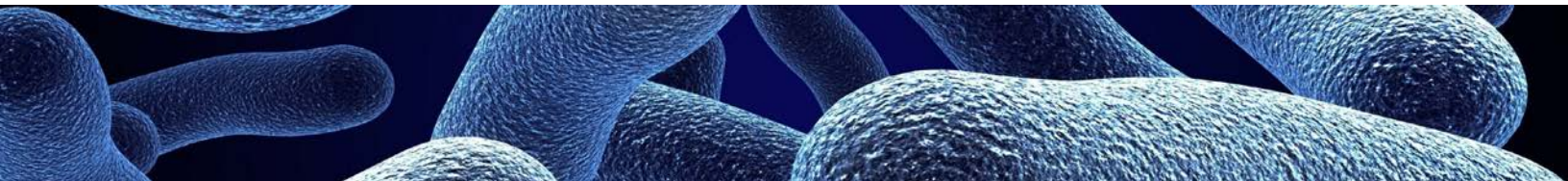


FIGURE 13: THE LYTIC AND LYSOGENIC CYCLE OF BACTERIOPHAGES. ADAPTED FROM [116].



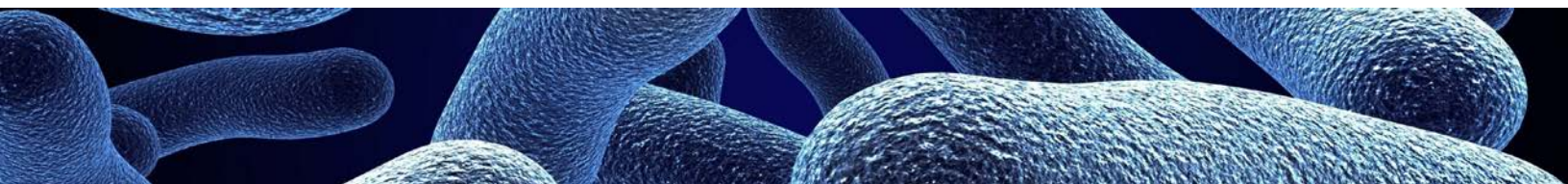
5.2.2.1. THE ROLE OF SYNTHETIC BIOLOGY IN PHAGE THERAPY

The increasing multi-drug-resistance of pathogens to existing antibiotics has revived the interest in alternatives for antibiotic use, among which bacteriophage therapy [117]. The advances in synthetic biology have given scientists the ability to design, modify and synthetize bacteriophages, enabling the engineering of new therapeutics, diagnostics or tools [6]. This way, functions can be added to the phages, or existing phages can be improved. Modifications to bacteriophages used to rely on random mutagenesis or homologous recombination. These methods are both time consuming and ineffective, as the mutants of interest have to undergo intensive screening. In addition, the genomes of bacteriophages are large and are inherently toxic to the bacterial hosts, causing difficulties in the use of conventional molecular biology techniques [6]. Synthetic biology tools however, have improved the ability to modify bacteriophages.

Using engineered bacteriophages, several strategies have been developed which can be categorized by their function. They aim at the use of phages to treat bacterial infections on their own (antibacterial phages), as well as using them as adjuvants, enhancing the effectiveness of current antibiotics (sensitization to antibiotics). Because of the high specificity of phages for bacteria, phages could be useful in the targeting of the harmful bacteria, while leaving the our normal bacterial gut flora intact [118]. In addition, because of this defined host specificity for a particular strain of bacteria, phages also show potential for detecting and typing of bacterial infections [119], this will be explained in chapter 5.6.2. The approaches using engineered bacteriophages targeting pathogenic bacteria are summarized in table 1. Here, two approaches of both categories will be explained to illustrate the concepts (*marked with an asterisk in table 1)*. A description of the remaining approaches can be found in Appendix I.4.

TABLE 1: SUMMARY OF THE APPROACHES USED WHEN ENGINEERING BACTERIOPHAGES. * THE APPROACHES EXPLAINED IN THIS CHAPTER.

Phages to sensitize the bacteria towards antibiotics	Antibacterial phages
Targeting non-essential genes and their networks*	Degrading bacterial biofilms*
Delivery of dominant antibiotic-sensitizing genes	Delivering a toxic payload*
Silencing antibiotic resistance with small-regulatory RNAs*	
Delivery of a CRISPR-Cas system	



5.2.2.2. SENSITIZATION TO ANTIBIOTICS

One of the ways engineered bacteriophages could be used, are as antibiotic adjuvants, sensitizing the bacteria for existing antibiotics. Indeed, the O'Neill Commission has stated that traditional antibiotics are still needed in the coming years, and the first wave of alternatives will probably serve as an adjunctive or preventive therapy [98]. Here, the phages are used as a chassis to generate a biochemical response to the antibiotics.

Targeting non-essential genes and their networks

Phages that are directly lethal to bacteria (lytic phages) can cause selection of phage-resistant bacteria and a release of toxic substances, such as endotoxins in the case of Gram-negative bacteria [112, 120]. One way to tackle this problem, relies on the targeting of the nonessential genes and their networks, that are not directly attacked by antibiotics. Lu and Collins [121] engineered a non-lytic bacteriophage to express LexA3, a repressor of the bacterial SOS response. This response is used by bacteria to counteract the oxidative stress resulting from the DNA damage caused by antibiotics (Figure 14). LexA3 was able to sensitize *E.coli* bacteria to three different types of antibiotics, namely quinolones, β-lactams and aminoglycosides. It decreased the survival of the *E.coli* bacteria both *in vitro* and *in vivo.* Although this method shows potential, it is still a proof-of-concept. Application in clinical practice still poses some barriers, such as phage immunogenicity, efficacy, and phage resistance [121]. However, some of these barriers can be overcome, such as the development of phage resistance by using phage cocktails. In addition, it does provide a platform on which phages can be engineered with different functions, targeting different bacteria.

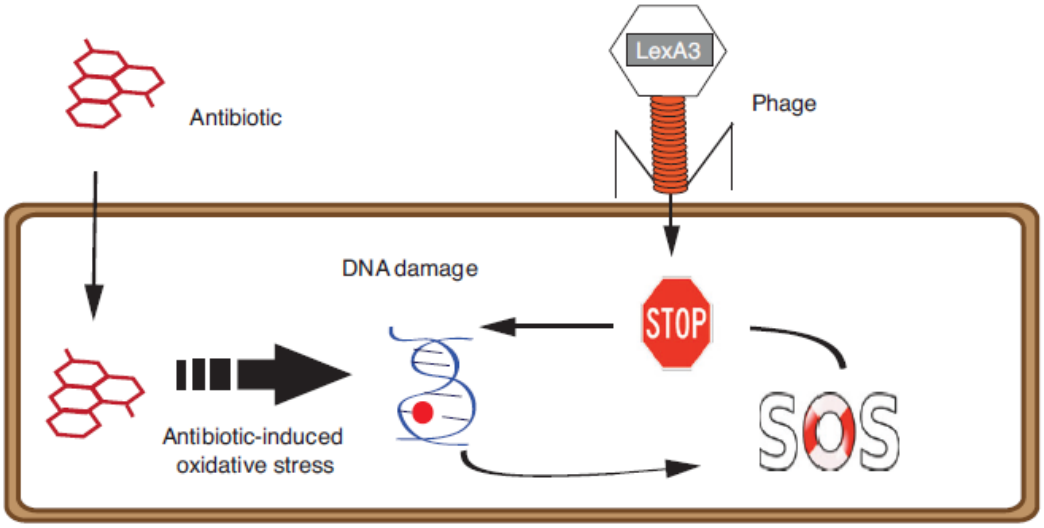
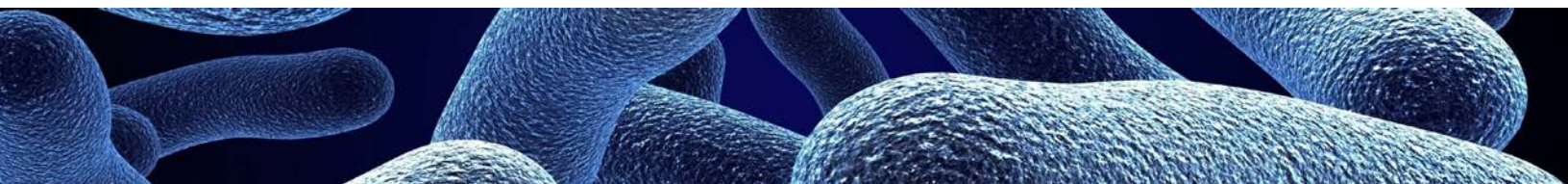


FIGURE 14: ENGINEERED PHAGES WERE USED TO REPRESS THE BACTERIAL SOS RESPONSE AND INCREASE SENSITIZATION TO ANTIBIOTICS. ADAPTED FROM [85].



Silencing antibiotic resistance with small regulatory RNAs

Another example of the engineering of phages, comes from Libis et al.[122], who also used non-lytic phages as a method of delivery. In their study, a bacteriophage was developed to knockdown the expression of two genes responsible for antibiotic resistance in *E.coli*. Small regulatory RNAs (sRNAs) were rationally designed to complement the antibiotic resistance mRNA and were transported towards the pathogenic bacteria via the bacteriophage. This way, these sRNAs targeted and silenced the mRNA, leading to antibiotic re-sensitization of the *E.coli* (Figure 15).

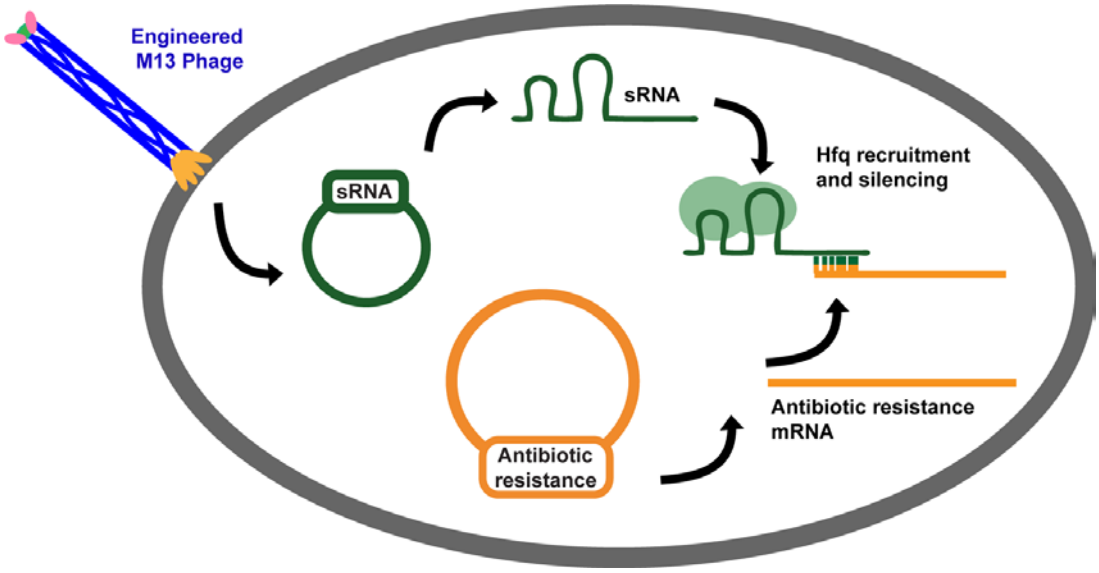


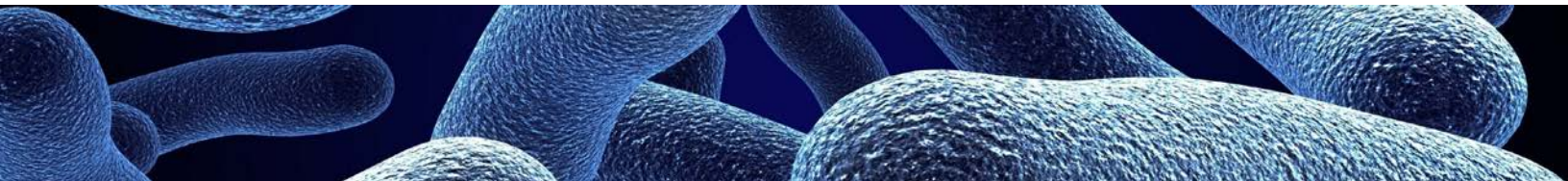
FIGURE 15: SILENCING OF ANTIBIOTIC RESISTANCE GENES TO RECOVER ANTIBIOTIC SUSCEPTIBILITY. ADAPTED FROM [122].

5.2.2.3. ANTIBACTERIAL BACTERIOPHAGES

The principles of synthetic biology can also be used engineer phages that directly target the pathogenic bacteria. This can, for example, be achieved via the degradation of the bacterial biofilm or the delivery of a certain toxic payload.

Degrading bacterial biofilm

As mentioned before, bacteria often live together in mixed communities called a biofilm. These biofilms shield the bacteria from antibiotics or the host immune systems, and play a critical role in their pathogenicity and infection [103]. In an early example, enzymatic bacteriophages were engineered to degrade bacterial biofilms and kill the bacteria residing in it [123]. Lu and Collins incorporated the gene encoding for Dispersin B, an enzyme able to enzymatically degrade the biofilm, into lytic bacteriophages. These modified phages were able to effectively degrade *E.coli* biofilms and expose the protected cells. These cells were again killed, resulting in a cycle in which 99,997% of the cells in the biofilm were removed (Figure 16). A limitation of this process, is the release of cytotoxic cellular components during the lysis of these bacteria, which could lead to an immune response [124].



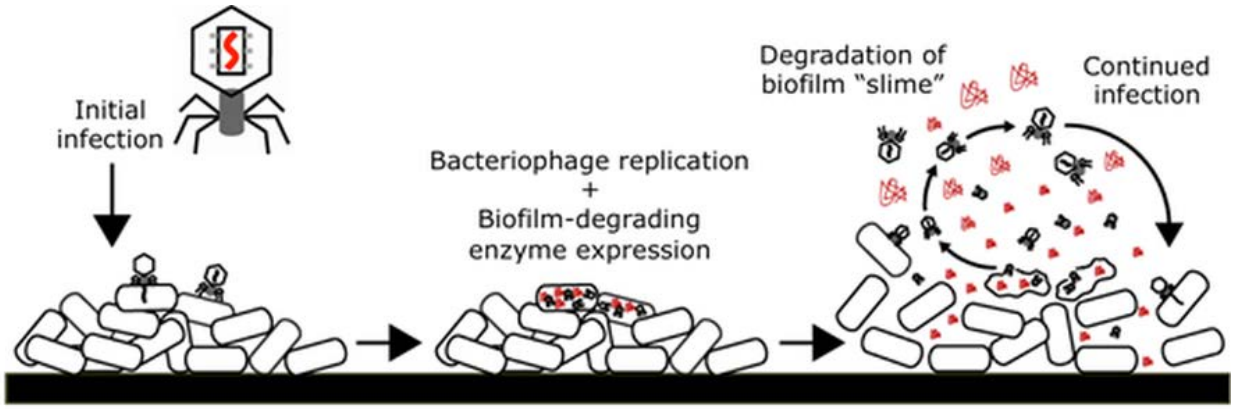
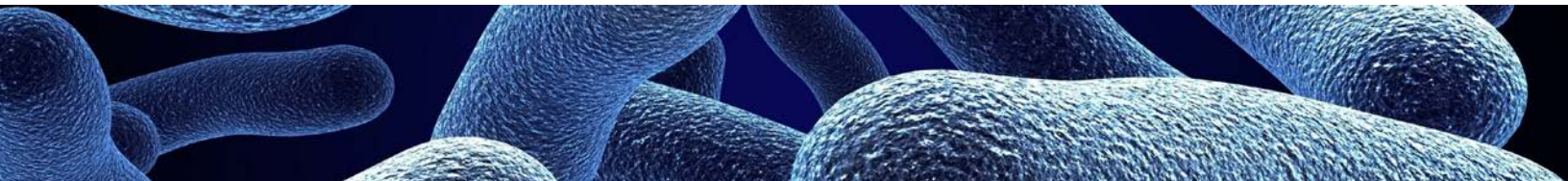


FIGURE 16: USE OF ENGINEERED BACTERIOPHAGES TO DEGRADE THE BACTERIAL BIOFILM. ADAPTED FROM [123]

These bacteriophages are currently being developed by the company EnBiotix (Brasil) Ltda. EnBiotix is an engineered antibiotics company developing novel systems and synthetic biology technologies. The phages are now being developed as an adjuvant to standard antibiotic therapy for the treatment of prosthetic joint infections[125]. In addition, together with Elanco Animal Health, the company is developing engineered phages as an alternative to traditional antibiotics in animals. Although the boundaries of the partnership exclude all non-animal health applications, it represents an important step forward [126].

Delivering a toxic payload

To prevent the release of the immunogenic cytotoxic cellular components, non-lytic phages can be used. Several groups used these genetically engineered phages to deliver a certain payload to bacteria. Westwater et al. [127] for example, used them to deliver the instructions (specific DNA) for cell-death to bacteria. This DNA encoded for so-called addiction toxins, which elicit destruction of the cells. Hagens et al.[128] on the other hand, used this concept to deliver genes encoding a restriction enzyme, which causes double-stranded breaks in the chromosome of the bacteria. This way, they increased survival rates of mice infected with *P.aeruginosa*, while also decreasing the amount of released endotoxin, which can lead to undesired side effects in phage therapy. Other genes that were brought into the target cells using engineered non-lytic phages encode the lethal catabolite gene activator (CAP) protein [129], the antibacterial protein SASP, which targets cells through non-specific binding to the DNA [130] and non-lytic antimicrobial peptides (AMPs) and toxin proteins, leading to non-lytic bacterial cell-death[131]. Thus, the engineered non-lytic phage can be used as a platform to deliver various types of payload to the pathogenic bacteria, providing a multifunctional tool in its killing or inhibition.



5.2.3. USING THE PRODUCTS OF PHAGES: LYSINS

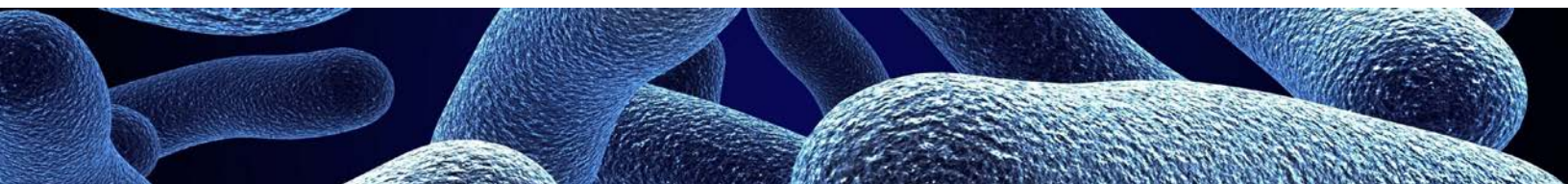
Together with the engineering of bacteriophages, synthetic biologists also use the modular nature of lysins to engineer them with enhanced functionalities. Endolysins, or lysins, are enzymes produced by bacteriophages. As mentioned, after the bacteriophage infects the host bacteria, a progeny of virion particles is produced. In order to release these virus particles into the environment, the cell wall of the bacteria has to be digested. This is achieved via the production of lysins, which are encoded in the bacteriophage DNA. They are usually bacterial genus-specific, thus leaving the commensal flora intact, and carry a low chance of the development of resistance against them. For these reasons, they are considered as a promising strategy against bacterial infections [132][Appendix II.3]

However, natural lysins are limited in their use for several reasons. Infections caused by mixed bacteria from multiple genera, cannot be treated with these lysins, as they are genus-specific [133]. In addition, natural lysins are generally only able to lyse Gram-positive bacteria, such as *Staphylococcus* and *Streptococcus* species. They are not able to pass the lipopolysaccharide layer surrounding the cell wall of Gram-negative bacteria [134].

In order to overcome these major hurdles, scientists are using the modular nature of lysins to engineer lysins with enhanced functionalities. Generally, lysins display a two domain modular structure: a catalytic domain that is responsible for degrading the cell wall, and a cell-binding domain that enables the recognition of the substrate [135]. This substrate is usually a unique and conserved molecule in the cell wall of bacteria, essential for its viability and restricted to a specific strain [133]. Shuffling these domains with a different origin, or fusing them with other molecules can create chimeric lysins or artificial lysins. These products have new and improved characteristics, such as a higher binding specificity, a broader spectrum or a higher activity [133]. Although some might argue that the engineering of lysins would be classified as protein engineering, this falls for many within the broad definition of synthetic biology.

ENGINEERING AN ENDOLYSIN

Lucacik et al. [1] engineered a hybrid lysin that can directly kill Gram-negative bacteria. The chimeric protein was composed of the translocation domain of the *Yersinia pestis* bacteriocin, called pesticin, and the enzymatic domain of a lysozyme from the *E.coli* phage T4 [6]. This product was both active against *E.coli* and *Y.pestis*, including those that normally conferred resistance against the unmodified natural bacteriocin.



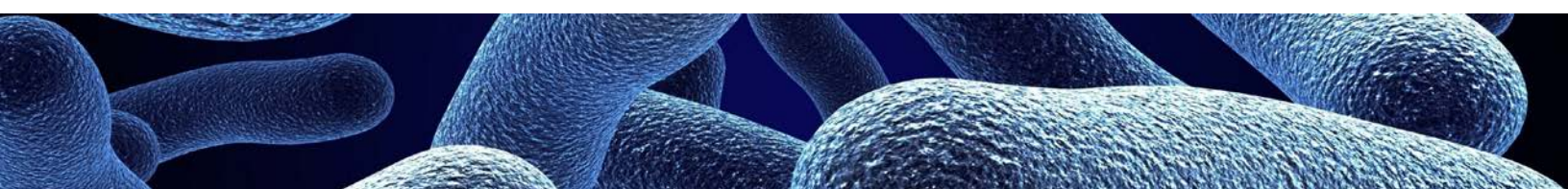
5.3. VACCINE DEVELOPMENT

To be able to use fewer antibiotics, it is also important to employ preventative strategies. One way of doing so, relies on the use of vaccines. Vaccines not only prevent viral infections, but are able to prevent bacterial infections as well. In addition, together with directly reducing antibiotic use, they can indirectly establish herd immunity to limit the level of transmission of pathogenic bacteria to susceptible individuals [136]. Currently, most vaccines are based on the inactivation of micro-organisms, the so-called live-attenuated vaccines, or on the use of subunits of purified components or recombinant proteins [137]. An indication of the potential benefit of vaccines to combat drug resistance has been estimated by the use of the pneumococcal conjugate vaccine. The WHO has estimated that *S. pneumoniae* kills over 800,000 children under five years of age every year. Global coverage with this vaccine could potentially prevent 11.4 million days of antibiotic use per year in these children, which corresponds with a 47% reduction [138, 139]. However, there are no licensed vaccines present for the three most urgent AMR threats stated by the US Centers for Disease Control and Prevention (CDC): carbapenemase-producing bacteria, drug-resistant gonorrhea, and *C.difficile* [140].

Therefore, novel vaccines are needed. Synthetic biology could contribute to their development in several ways. So far however, most synbio research has focused on the production of vaccines for viruses, while less has been done in order to produce vaccines towards bacteria [93]. One example of the focus on viruses, is the identification of new protein vaccine candidates using computer-based screening algorithms, or the improvement of the expression of naturally occurring antigens [141].

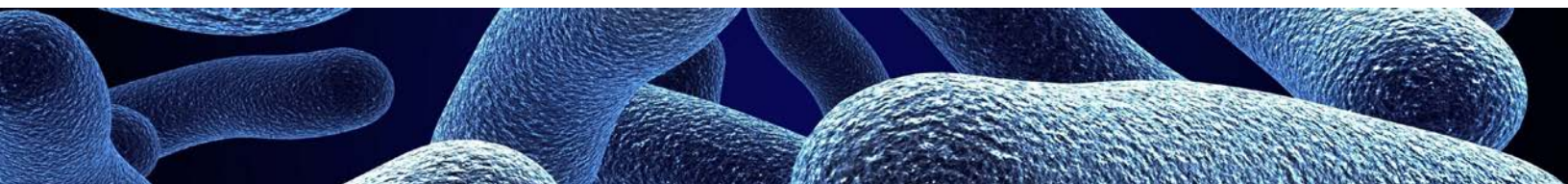
Another example is the production of an influenza vaccine using a synbio approach. Usually, influenza viruses are collected and shipped to qualified centers, where they are co-cultured in eggs and the genetic material is mixed into new combinations. These reassortments are then sent to vaccine manufacturers [142]. In 2013, the first vaccine developed using synthetic biology, was tested in humans. On 24 April 2013, the discovery of a new, potentially pandemic, strain of avian influenza was found in China. The Chinese CDC made the sequence of the haemagglutinin (HA) and neuraminidase (NA) antigens from this virus available online. The laboratory of Craig Venter synthesized the DNA encoding these antigens and the plasmids encoding the other RNA segments of the virus the next day [143]. The virus that was generated from these synthetic genes was then used as a seed in the manufacturing of a subunit vaccine and tested in a Phase I clinical trial, showing promising results [144]. Thus, synbio provided an opportunity to accelerate vaccine availability in the case of a pandemic [142].

Another approach, in which so-called ‘smart vaccines’ are created, has also been suggested [145]. These vaccines are based on RNA circuits, rather than circuits encoded by DNA. DNA-based delivery platforms in mammalian cells have many limitations, including vector integration, causing a possible mutation of the host genome. In addition, the DNA construct can be epigenetically silenced, and problems can arise in the gene expression of non- or slowly dividing cells [145, 146].



mRNA-based approaches on the other hand, have become more popular. They carry a very low risk of mutagenesis and can cause an immediate expression of the protein of interest, even in non-dividing cells [147]. While RNA-based vaccines have several benefits, such as better compositional control and a lower cost of production, their creation is still challenging [145]. In their paper, Andries et al. have suggested ideas for these 'smart vaccines' with programmable RNA circuits. These vaccines would also be beneficial for communities with limited access to vaccination clinics, as the vaccines would be engineered to offer one-shot injections, not requiring the normal 'booster' shots [145].

One example of a company developing a vaccine using synthetic biology, is Prokarium. In 2015, they received €535.000 from SynbiCITE, the UK's Innovation and Knowledge Centre for Synthetic Biology to develop a Chlamydia vaccine. An engineered strain of *Salmonella* containing the blueprint for the vaccine functions as a carrier, which is taken orally. It enters then through the gut lining, and it naturally engulfed by the body's immune cells. Only then is is triggered to produce the active vaccine [148, 149]. This method can also be seen as a method of targeted therapeutic delivery, such as the engineered probiotic bacteria that have been described before.



5.4. DIAGNOSTICS

5.4.1. THE IMPORTANCE OF POINT-OF-CARE DIAGNOSTICS

Finding new antibiotics or an alternative will not solve the problem on its own. As any use of antibiotics encourages resistance, it is of great importance that we limit their unnecessary use [150]. In order to do so, doctors have to be able to rule bacterial infection in or out. In addition, diagnostics should be able to tell to which antibiotic the infection is susceptible. Synthetic biology offer tools to create new diagnostics, possibly faster and cheaper than current diagnostics.

An example of the problem of unnecessary use due to the lack of rapid diagnostics, is given in an academic study published in 2013 in the *Journal of Antimicrobial Chemotherapy [151].* In America, around 40 million people are given antibiotics for respiratory infections every year. 27 million of which received an antibiotic although they may have not needed it, for example because they were thought to have respiratory issues that antibiotics cannot solve, such as asthma or bronchitis. This is part due to the prescribing habits or doctors, or because of the expectations of patients. It is also more expensive and more time-consuming to use a diagnostic that using an antibiotic just in case [150]. Antibiotics are being reimbursed in our current healthcare system, while diagnostics will have to be paid by the doctor [Appendix II.8]. In addition, the commercial interest of pharmaceutical companies for the development of rapid diagnostics is low, as their use would limit the amount of antibiotics used [150]. Although there is a need for different economic models and behavioral changes, the development of these rapid diagnostics remains essential as well. According to the O'Neill Commission, the perfect new diagnostic test would answer the following four questions (Figure 17):

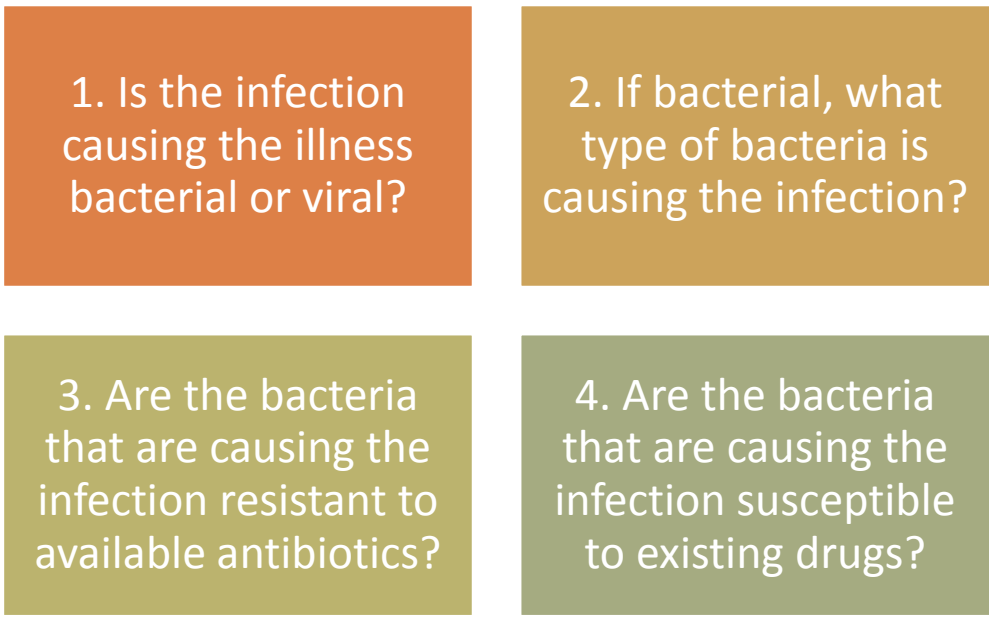
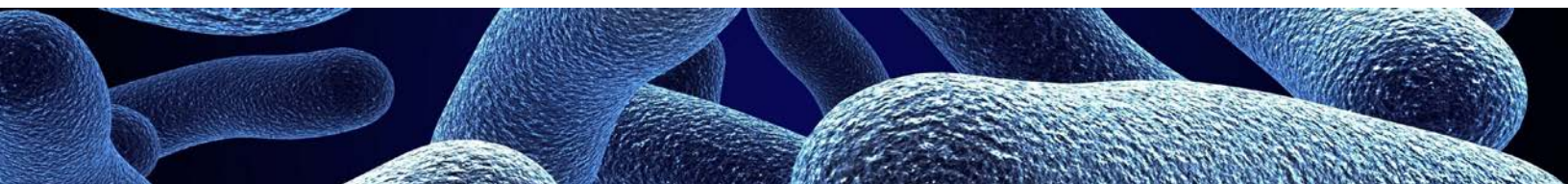


FIGURE 17: THE FOUR QUESTIONS THAT NEED TO BE ANSWERED BY THE PERFECT NEW DIAGNOSTIC TEST, ACCORDING TO THE O'NEILL COMMISSION [150].



Currently, diagnostic tests can answer these questions, but the process is slow. Lab tests, in which the bacterium has to be grown, take up to 36 hours. In many situations, by then the treatment has already started, possibly with the wrong drugs. In addition, this process increases the number of pathogens, which could be a source of concern [152] Synthetic biology provides some tools for these developments, including the use of engineered bacteriophages and so-called paper-based diagnostics.

5.4.2. PATHOGEN DETECTION USING IMPROVED BACTERIOPHAGES

Several studies have shown the ability of engineered bacteriophages to destroy bacterial biofilms, increase the efficacy of existing antibiotics or to have a bactericidal effect themselves. However, bacteriophages can also be engineered to function as a diagnostic tool, by transforming them into factories for detectable molecules. And while therapeutic bacteriophages are subjected to strict regulations, their use as diagnostics is already having success the food-industry or in laboratory settings [124, 152].

As mentioned, bacteriophages have a natural specificity for bacterial types, which is referred to as their host range. Only live bacteria are infected (and thus detected), because phages need the energy of the host cells for replication [153]. These abilities have been used for decades in order to identify target bacteria, a process called phage typing. Unknown bacteria were cultured and small drops of different phage solutions were added. When the bacteria were lysed, or plaque occurred, it could be noted which phage infected the bacterium [153]. To create a better signals, and shorten the time required to obtain a readable output, these phages can be genetically engineered [124, 152]. This way, fluorescent, bioluminescent or colorimetric output can be created, or identification can take place of as few as ten bacterial cells per milliliter per hour [124, 154-157].

5.4.3. PAPER-BASED DIAGNOSTICS

Although different types of sensors have been developed that are based on either DNA, or RNA, they all require a cellular context. In order to overcome this challenge, cell-free systems have been designed. These systems consist of the transcription and translation machinery, an energy source and the DNA constructs of interest. However, these systems are still solution-based and are submitted to strict protocols [158]. Pardee et al. therefore developed a paper-based diagnostic system, in which the gene-expression machinery, along with the gene-circuits is embedded onto paper via freeze-drying [159](Figure 18).

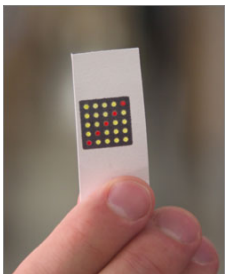
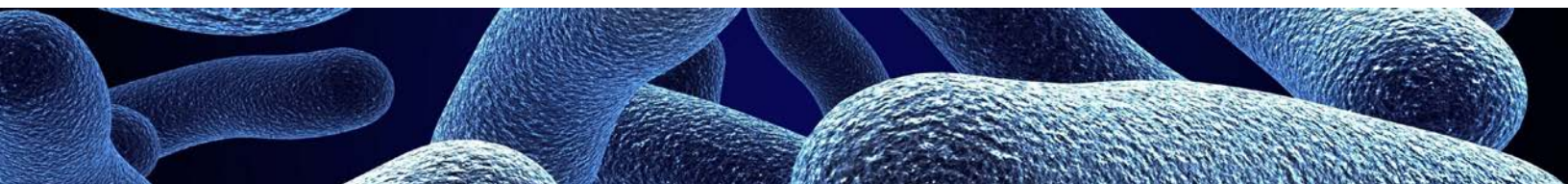


FIGURE 18: PAPER-BASED DIAGNOSTICS [12].

It functions using a so-called RNA toehold switch, regarded as a major innovation on its own in synthetic biology [160]. In this toehold switch, a ribosome-binding site (RBS) (at which the ribosome binds and translation follows) is placed within a hairpin. Gene expression is activated when trigger RNA unwinds this hairpin and exposes the RBS (Figure 19). Importantly, this trigger RNA can be virtually any sequence, expanding the diversity of target sequences greatly. [158].



Upon activation of gene expression, a colorimetric output follows. This way, different bacterial RNA can be identified simply by a change of color on the paper. This system was demonstrated by the authors of the paper by developing 24 mRNA sensors for Ebola viruses in less than a day. It was able to rapidly detect and distinguish between two different strains of Ebola from the 2014 outbreak [124, 158, 159]. It represents a low cost, and portable method which can, due to its modular nature, easily be scaled up. In addition, paper can be patterned into different regions, providing the screening of multiple samples on one piece of paper [159].

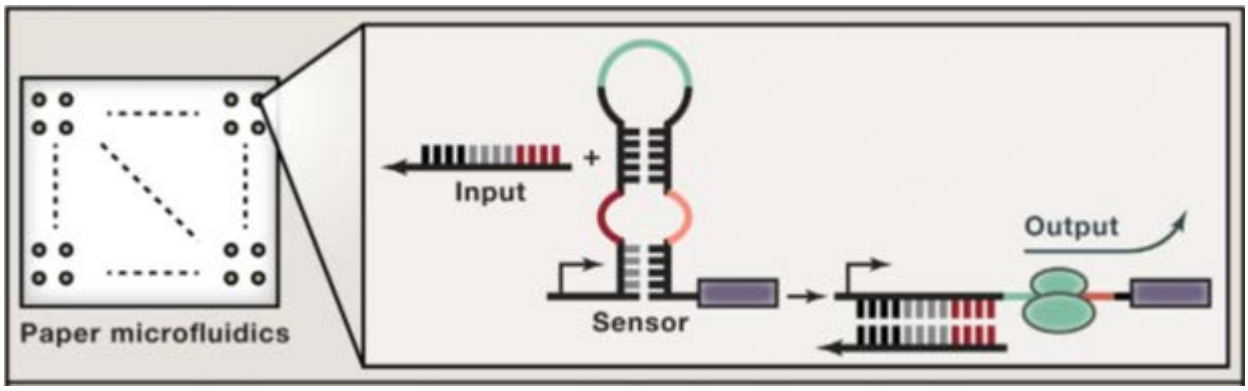
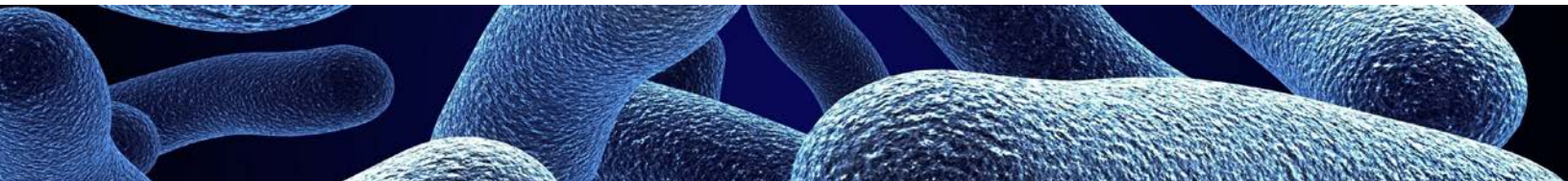


FIGURE 19: REPRESENTATION OF A PAPER-BASED TOEHOLD SWITCH. ADAPTED FROM [158].



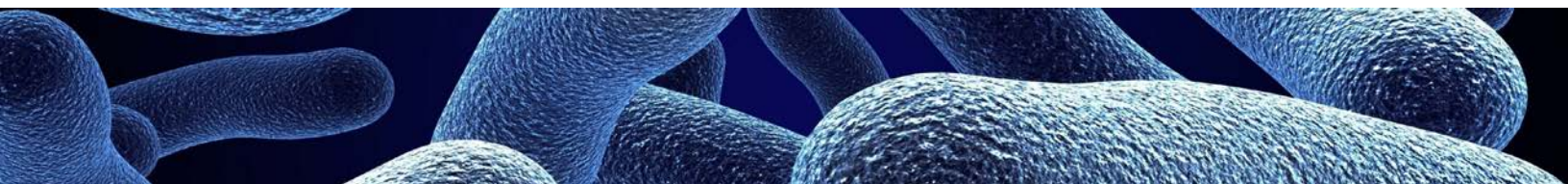
5.5. CONCLUSION

AMR poses a significant health problem to society. Although it is a multi-faceted and complex problem, one of the causes is the lack of new antibiotics, alternatives and appropriate diagnostics. One the other hand, synthetic biology is an emerging field, in which various developments seem to offer new approaches towards tackling AMR.

The developments mentioned in this chapter are summarized here in Table 2. In general, synthetic biology seems to offer a platform, and is not limited to a single type of development. Although the developments mentioned in this chapter are generally still far from licensed and clinically approved products, the field is increasing in popularity. The fact that DNA synthesis is only getting cheaper, our knowledge of gene sequences is increasing, and new gene-editing techniques are on the rise, only adds to this popularity. It is therefore of importance to stay up-to-date with these and other developments, in order to identify chances and barriers, and minimize these in advance.

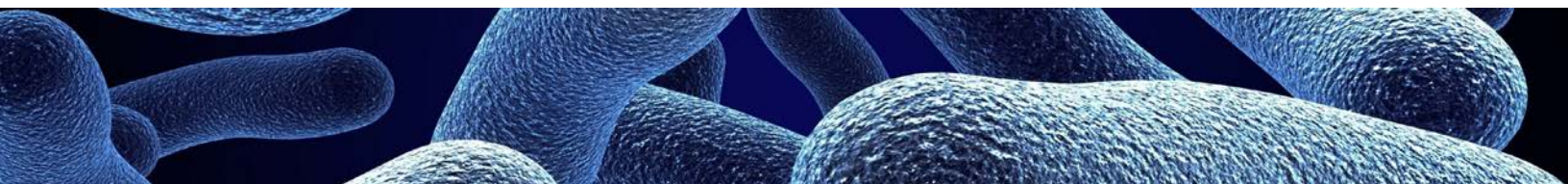
TABLE 2: SUMMARY OF AMR/SYNBIO DEVELOPMENTS

Novel antibiotics	Alternatives to antibiotics	Diagnostics
Target identification using the minimal genome approach	Engineered bacteriophages	Pathogen detection using improved bacteriophages
Screening platforms	Engineered probiotics	Paper-based diagnostics
Discovery of novel natural antibiotics by awakening 'silent' BCGs	Engineered lysins	
Creating new-to-nature molecules	Accelerate vaccine availability/creating 'smart' vaccines	



Part 2

The role of the government in stimulating (synbio) innovation: some considerations.



CHAPTER 6: INTRODUCTION

6.1. AIM

The aim of part two of this report is to answer the following research question:

What considerations can be made by the government upon deciding on their role in stimulating (synbio) innovation in AMR?

In part one, the developments in synthetic biology that could contribute to tackling AMR are summarized. These can either aid in the development of new antibiotics, alternatives to antibiotics, or diagnostics. Therefore, although AMR is a complex problem for which several solutions are necessary, specifically the role of innovation is here considered. The role of the government in AMR in general, and in stimulating innovation, can also be manifold. In order to make decisions regarding these different roles, it is important to consider the different aspects that come into play. To this end, an assessment of the policy and research framework has been made, and viewpoints of stakeholders within these frameworks on the role of the government have been actively considered. This part provides a first analysis of the results, leading to several considerations.

6.2. APPROACH

A simple linear model of the antibiotic innovation pipeline

To identify the different aspects that come into play, the events or processes that take place in AMR innovation have been structured. Although the focus here will be mainly on innovation regarding new antibiotics (or alternatives), the role of diagnostics will also be touched upon. For practical purposes, I have adopted a simple linear model of events that represents the antibiotic innovation chain or pipeline (mainly focusing on public stakeholders and processes) (Figure 20).

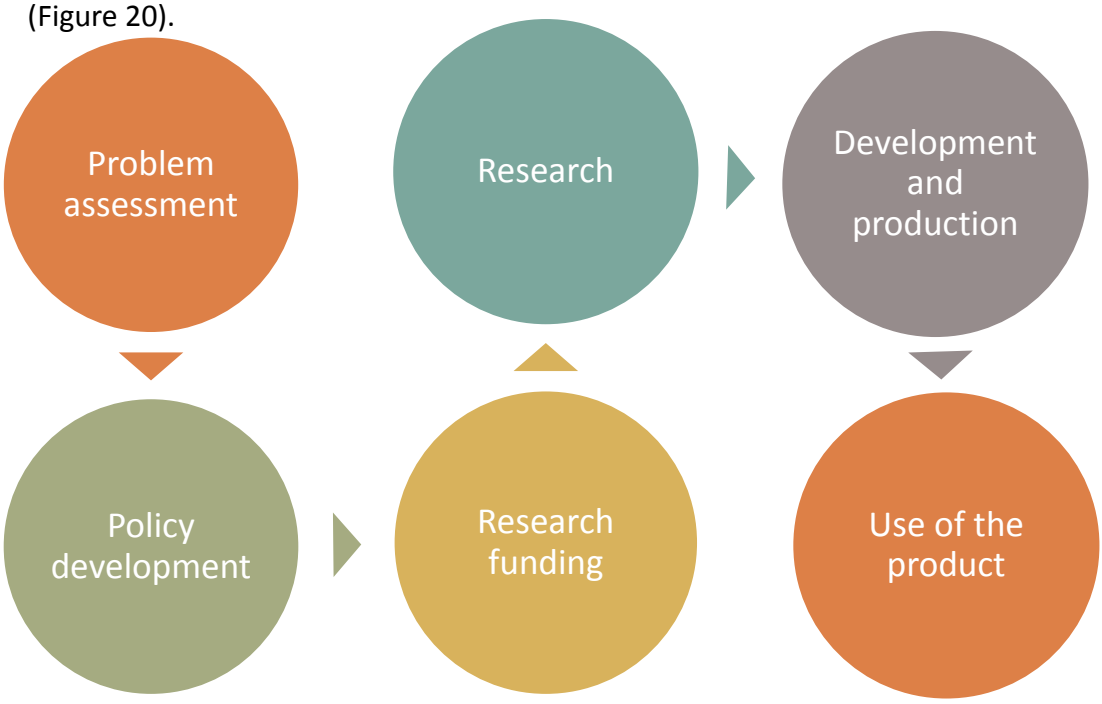
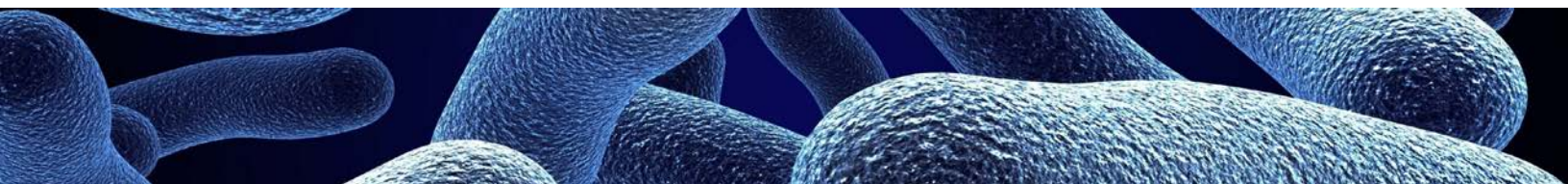


FIGURE 20: A SIMPLIFIED LINEAR MODEL OF THE ANTIBIOTIC INNOVATION PIPELINE, BASED ON AN ASSESSMENT OF THE PROBLEM.



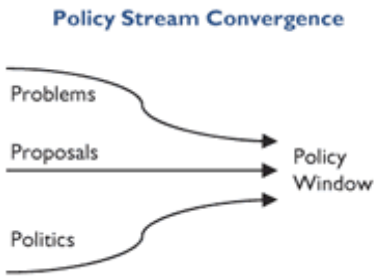
Problem assessment: The model starts with an assessment of a part of the AMR problem: the scarcity in the development of new antibiotics, alternatives to antibiotics and appropriate diagnostics. To solve this problem in a political sense, it is however not enough to simply acknowledge a problem, the conditions have to be right. Or, when referring to Kingdon's streams model, all three streams (problem, policy and political) will have to come together for a problem to be set on the political agenda *(See text box 'Kingdon's streams model').*

KINGDON'S STREAMS MODEL

According to Kingdon, three separate 'streams' need to come together for an issue to be put on the political agenda:

1. Problem stream: attention is captured towards a problem; the problem is recognized.
2. Policy stream: regards proposals for change (solutions to the problem).
3. Politics stream: policymakers have the motive and opportunity to turn this into policy.

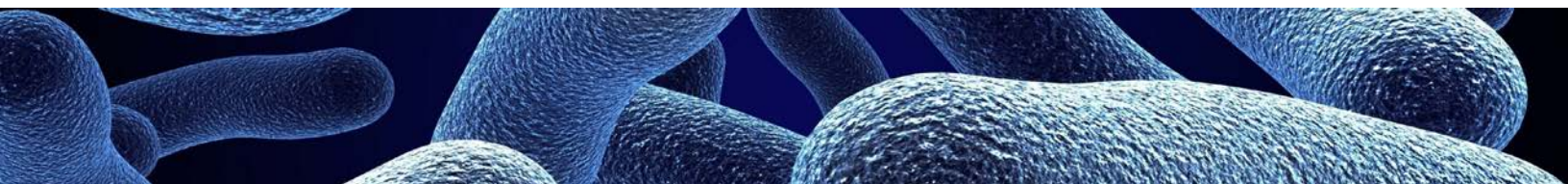
When these three streams come together, it creates a *window of opportunity.*



Policy development: After a problem has been set on the agenda, a policy is developed. To tackle AMR, and to stimulate antibiotic innovation, policies on different levels have been developed. These will be described in more detail in chapter 7. In short, these policies can offer different types of incentives to stimulate antibiotic research and development: pull- and push-incentives. Pull mechanisms reward successful development of a drug by increasing or ensuring future revenue. These can be outcome-based, such as monetary prizes or patent buyouts, or focused on regulatory chances, such as accelerated drug approval and value-based reimbursement. Push mechanisms on the other hand reduce the cost of researching and developing new drugs. This includes providing research grants or establishing public-private partnerships [57].

Research funding: Here, the main focus will lie on the provision of research grants, with which research towards the developments of novel antibiotics, alternatives, or diagnostics can be stimulated. These grants are divided by funding organization(s), via a process involving various calls and assessments.

Research: Both public and private AMR research is being done, in various research areas, among which is synthetic biology. In this model, the focus is mostly on public research by knowledge institutions. It is noted that the specific area of synthetic biology that focuses on providing developments towards tackling AMR, is quite small in the Netherlands.



Development and production: If the research outcomes are successful, they are generally transferred to businesses (a process called technology transfer). This is mainly due to the limited abilities of universities to develop these outcomes further [Appendix II.8]. Either small- and medium enterprises (SMEs) or large pharmaceutical companies will further develop and produce the new antibiotic.

Use of the product: The new antibiotic (or diagnostic) is used by medical professionals in the treatment of (resistant) bacterial infections in patients.

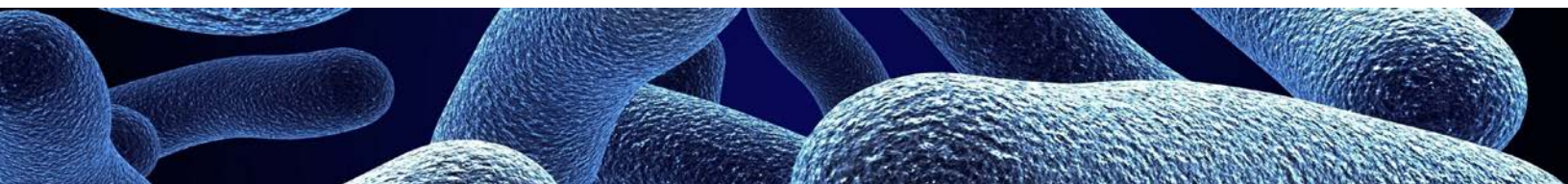
Stakeholders in the antibiotic innovation pipeline

In these processes, different stakeholders are involved. To assess their different roles and arguments on the role of the government in AMR innovation, interviews have been held per link of the innovation chain (Table 3) *(For the list of interview participants and their function, see appendix II.1.)*. However, the main focus of these interviews was on the first part of the innovation pipeline. It therefore has to be noted that interviews held to obtain insights into arguments and viewpoints in the development and production process, were held with either an investment company, or a network organization. Both organizations represent stakeholders involved in the innovation pipeline that can offer insights into considerations made by businesses.

Three types of interviews were held:

1. Introductory/exploratory interviews.
2. Semi-structured interviews.
3. An interview for critical reflection of these answers given in other interviews.

In addition, a structured questionnaire with both open and closed questions was used to gather the opinion of scientists (and other stakeholders present at the symposium) on the viability of the current developments of new antibiotics and alternatives to antibiotics (Appendix IV). The questionnaire participants were also asked for their viewpoint on the role of several stakeholders in stimulating AMR innovation, and on the means necessary to do so.

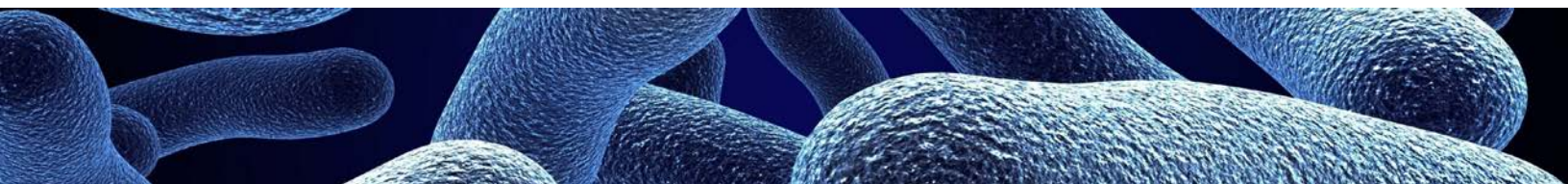


Process	Identified type of organization	Representative actor
Problem assessment and policy development	Ministry of VWS	Policy makers (2)
Research funding	ZonMw	Program leader ABR ZonMw
Research	Knowledge institutions	(Synbio) scientists (3)
Development and production	Network organization and investment company	Managing director of a network organization and an investment professional
Use of the product	University hospitals	Head medical microbiology (2)

TABLE 3: INTERVIEWS WERE HELD WITH STAKEHOLDERS AT SEVERAL POINTS IN THE INNOVATION PIPELINE.

6.3 READING GUIDE

To create insight and to be able to interpret the results of the interviews and questionnaire, the current AMR policy regarding the stimulation of innovation (including the funding policy of ZonMw) has been outlined. The AMR policy framework, with a focus on innovative incentives, will be described on global, European and national level in chapter 7. In chapter 8, the policy of ZonMw with regard to the division of research funding will be described. Chapter 9 will provide an overview, which aids in the understanding of the processes and actors involved in later stages of the innovation pipeline (development and production). The outcomes of the interviews and the questionnaire will be described, discussed and analyzed in chapter 10. Finally, chapter 11 will provide the reader with conclusions on both part one and part 2 of this report, while also offering recommendations.



CHAPTER 7 – AMR: CURRENT POLICY FRAMEWORK

7.1. INTRODUCTION

The acknowledgement of the AMR problem has led to the development of several policies, globally, European and national, in order to combat AMR. In general, they all focus on promoting a multi-lateral, global and inter-disciplinary approach. AMR is a complex and multi-faceted problem and cannot be solved simply by using fewer antibiotics, discovering and producing new antibiotics, or good hygiene alone. In addition, is it not confined to the human world, but affects animals, the environment and our food as well. Therefore, a plethora of measures to tackle AMR has been stated in several reports and policy documents. The current policies described in this chapter are based of former policies that have been designed, evaluated and redesigned. The initiatives following these policies are being implemented, and have to be evaluated as well. The results in part two of the report could be used to aid in this process (Figure 21).

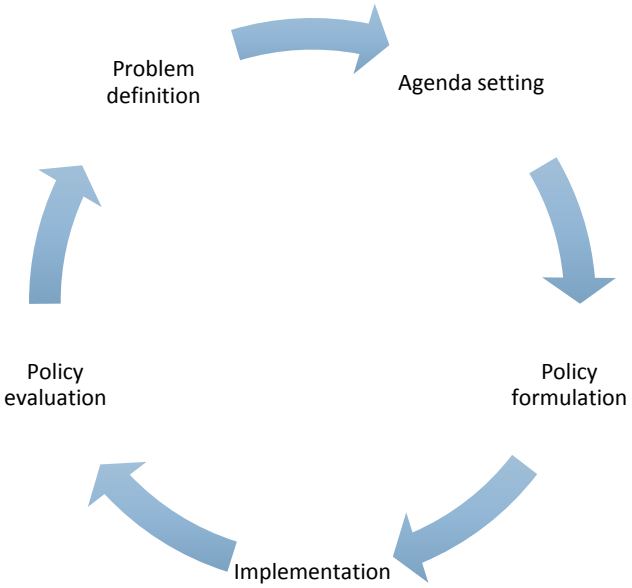


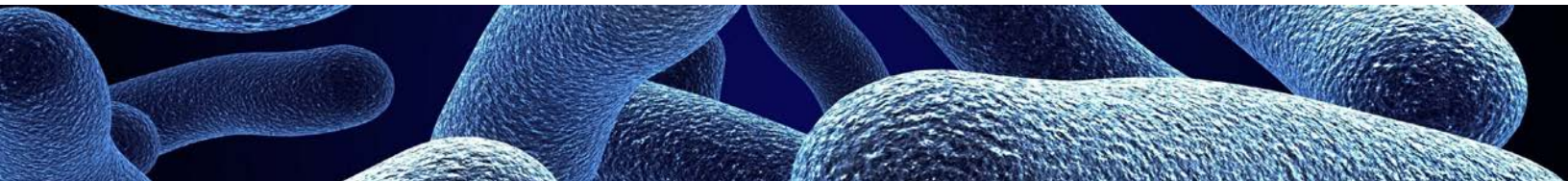
FIGURE 21: AMR POLICY CYCLE. THE PROBLEM HAS BEEN ACKNOWLEDGED EARLY ON, AND POLICIES HAVE BEEN FORMULATED, IMPLEMENTED, EVALUATED AND ADAPTED.

Here, the focus will be on the stimulation of AMR innovation in the global, European and Dutch national AMR policy. In these policies, a focus on innovation has always existed, as the lack of new antibiotics with a new working mechanism has been acknowledged for many years. Therefore, several initiatives have followed and were implemented, targeting antibiotic innovation.

'Innovation' [11]:

1: the introduction of something new.

2: a new idea, method, or device

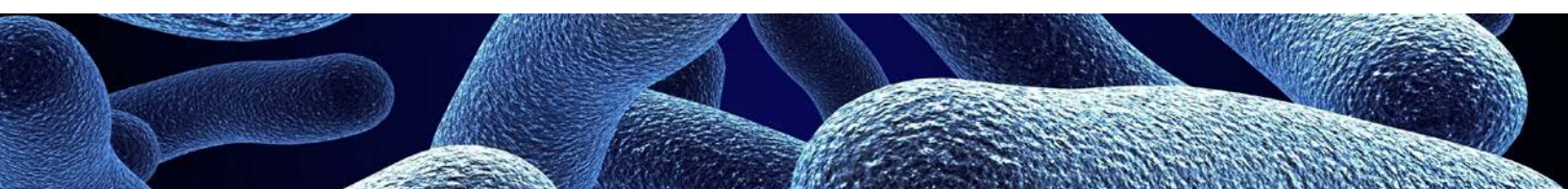


These are summarized in Table 4, and a more detailed description can be found in the report on 'International and European Initiatives Targeting Innovation in Antibiotic Drug Discovery and Development' [57]. The initiatives are aimed at both the development of knowledge (for example about resistance mechanisms) and the development of new antibiotics, alternatives or diagnostics. Below, some of these initiatives will be explained shortly.

The initiatives are also getting more and more aligned. Currently, the JPIAMR Alignment Plan sets the basis to align national and European research policies in the area of AMR during the period 2014-2018. This is done in order to enable the research priorities, outlined in the SRA-JPIAMR, to be addressed [161]. Through adoption of the global action plan of the WHO, governments are all committed to have in place, by May 2017, a national action plan on AMR that is aligned with the global action plan[162].

TABLE 4: GLOBAL, EUROPEAN AND DUTCH ANTIBIOTIC R&D INITIATIVES [57].

Global
WHO Global Action Plan AMR
Transatlantic Taskforce on AMR (TATFAR)
Global Health Security Agenda
G7 Global Union for Antibiotics Research and Development (GUARD)
European & Developing Countries Clinical Trials Partnership (EDCTP)
EU
EU Antimicrobial Resistance Action Plan
The European Commission's Directorate-General for Research and Innovation(DG-RTD)
Innovative Medicines Initiative (IMI) – New Drugs for Bad Bugs (ND4BB)
InnovFinn Infectious Disease Finance Facility (InnovFinn IDFF)
European Medicines Agency (EMA)
The Netherlands
Netherlands Centre for One Health (NCOH)
Netherlands Organization for Health Research and Development (ZonMw)



7.2. GLOBAL AND EUROPEAN POLICY ON AMR

7.2.1. GLOBAL AMR POLICY

The World Health Organization (WHO) has identified AMR as one of the greatest current threats to global health [53]. Antibiotic resistance is a global problem, as bacteria are not affected by boundaries. International cooperation can aid the efficiency of measures and (research) investments [202]. Therefore, in September 2001, the first WHO Global Strategy for Containment of Antimicrobial Resistance was issued. Another initiative followed, as well as several other cooperations and agreements. A short summary of some of these initiatives is given below (Figure 22).

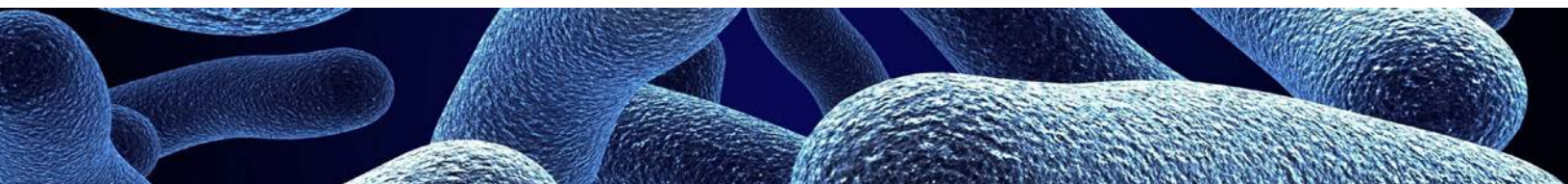
The *second WHO Global Action Plan Antibiotic Resistance* was adopted in May 2015, during the annual meeting of the World Health Organization (WHO)[203]. The Dutch Government supports the implementation of this plan in several ways, including a financial contribution of VWS and strategic secondments at the WHO.

The *Transatlantic Taskforce on Antimicrobial Resistance (TATFAR*) was established in 2009, in response to the mounting threat of antimicrobial resistance. It aims to identify urgent AMR issues and propose recommendations to combat these issues [202]. Their recommendations focus on three tasks: appropriate use, prevention, and preservation and innovation. Although TATFAR does not offer any direct incentives towards innovation, it does ensure good a cooperation between government agencies involved in this process (funding, drug approval and market policies and regulations)[8].

The Global Health Security Agenda, initiated by the United States in February 2014, is a partnership of nations, international organizations, and non-governmental stakeholders. The agenda exists out of eleven 'action packages'. The Netherlands is active on two of them: antibiotic resistance and zoonosis. These efforts are mainly focused on strengthening and coordination of technical support to countries that are in need of this [203].

The Global Union for Antibiotics Research and Development Initiative (GUARD) is an agreement between the G7 nations made in 2015 [7*].* It states that a collaborative approach between nations is needed, and proposes priority areas for action and recommendations to stimulate antibiotic research and development. However, no concrete incentives have followed so far [8]

FIGURE 22: DESCRIPTION OF SOME OF THE GLOBAL AMR INITIATIVES.



7.2.2. EUROPEAN AMR POLICY

In the Dutch presidency of the EU in the first half year of 2016, antibiotic resistance was a key focus point. During an informal ministerial conference on February 10th 2016, for which both the EU ministers of Health and of Agriculture were invited, the evaluation of the current EU Antimicrobial Resistance Action plan was presented. This plan was drafted in 2011 and will end in 2016. Several preliminary key achievements were detected, as well as lessons for the future. Whereas considerable progress has been achieved in supporting research and innovation, it still needs to be promoted. The development of antimicrobials, rapid diagnostics tests, vaccines and alternative treatments remains important for the future [163]

Several European initiatives already exist in order to stimulate these developments. The European Commission has formed three key pillars regarding innovation in its strategy to combat AMR, described below [57] (Figure 23).

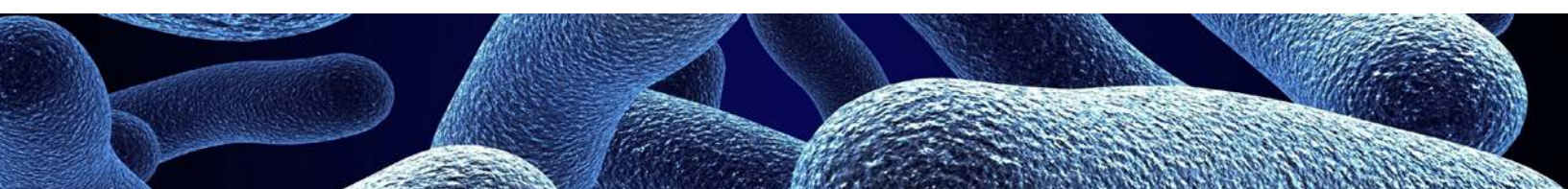
The Innovative Medicines Initiative (IMI) is an initiative between the European Commission and the private sector. Their New Drugs for Bad Bugs (ND4BB) program was started in 2012 and aims to improve the discovery and development of new antibiotics.

The European Joint Programming Initiative on Antimicrobial Resistance (JPIAMR) aims to improve the cooperation between EU member states (as well as partner states such as Canada) on AMR research. It does so by tuning national research programs and the joint programming and financing of costly and complex research of academic groups.

The European & Developing Countries Clinical Trials Partnership was formed in 2003 and is a public-private partnership between European and African countires, NGOs, and pharmaceutical companies. It aims to enable research collaboration and accelerate the clinical development of drugs for neglected infectious diseases .

FIGURE 23: DESCRIPTION OF SOME OF THE EUROPEAN AMR INITIATIVES.

In addition, the European Framework Programme for Research and Innovation exists, also known as Horizon 2020. Within this program, the InnovFin Infectious Disease Finance Facility (IDFF) offers loans to companies to progress their medical products (that combat infectious diseases) through the clinical stages [164].



7.3. AMR POLICY IN THE NETHERLANDS

In the letter to Parliament of July 2nd 2013 [165], the Minister of Health, Welfare and Sport acknowledges the lack of the development of new antibiotics. In addition, it is mentioned that generally, the development of new drugs is primarily the responsibility of the pharmaceutical industry. However, if the stimuli from the market to develop these new drugs are insufficient, for example because of high risks or low profit margins, a role for the government in this development is also present.

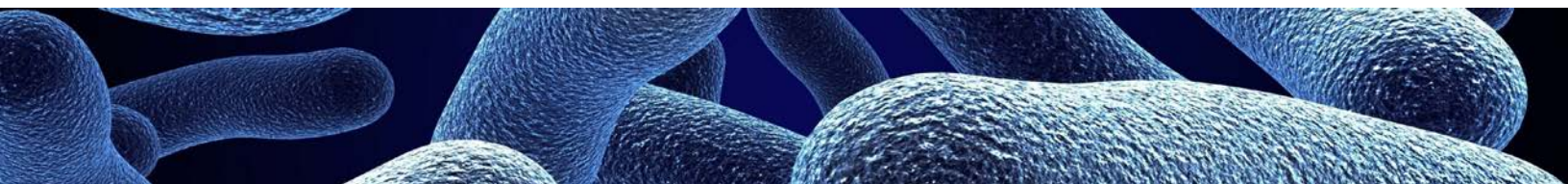
In this letter to Parliament, emphasis is also placed on the fact that one country alone is not capable of this, and an international approach is needed.

> "It is the role of the government to create an environment for private investors, as such that the public interests are safeguarded. This can include restrictions in terms of safety, but also stimulatory measures when private investments are socially suboptimal." [5, 6, 8]
>
> Netherlands Scientific Council for Government Policy (WRR).

7.3.1. THE CURRENT STATUS

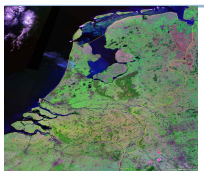
An outlined multifactorial approach of these intentions has been given in the letter to Parliament of June 24th 2015, aimed at the period 2015-2018 [166]. Innovation is seen as an essential prerequisite in order to combat antimicrobial resistance effectively. Not only is the development of new antibiotics of importance, but improved infection prevention as well. In addition, counteracting the spread of resistant bacteria, an improved application and quicker diagnostics, and alternative treatments are important.

In the letter, several signals are stated. First, the urgency to produce new and improved antibiotics, is acknowledged by all relevant Dutch stakeholders, such as universities, pharmaceutical companies and knowledge institutions. Second, The Netherlands has a good reputation when it comes to research on infectious diseases and a good infrastructure to stimulate innovation exists. However, cooperation between the different research groups and the research climate in the Netherlands is not seen as optimal, and thus needs to be strengthened. In addition, fundamental and translational research towards new antibiotics and alternatives will require more attention. The subsequent clinical research is seen as expensive and lengthy. Lastly, the current business model for developing new antibiotics is characterized by an insufficient suspected 'return of investment'. The new products that are developed by companies, are used as little as possible, or preferably not at all, in order to prevent the development of resistance against them. This causes the possibilities to invest in these development to be absent.



7.3.2. AN OUTLINED APPROACH

Therefore, the following approach is suggested in this letter (Figure 24):



Cooperation between research institutions and companies needs to be strengthened, via a *joint national research agenda.* The strengthened research infrastructure will lead to an improved network, in which knowledge is shared and fundamental, translational and clinical research will come together, in order to enhance the link between all steps in the innovation chain. Research results will, if possible, be used in public-private partnerships to continue its development. To enhance and strengthen the research infrastructure even more, connections will be made with ongoing initiatives, research institutes and knowledge centers, as well as the top sectors Life Sciences &Health (LSH), Chemistry and Agro. This has lead to the founding of the *Netherlands Centre for One Health.*

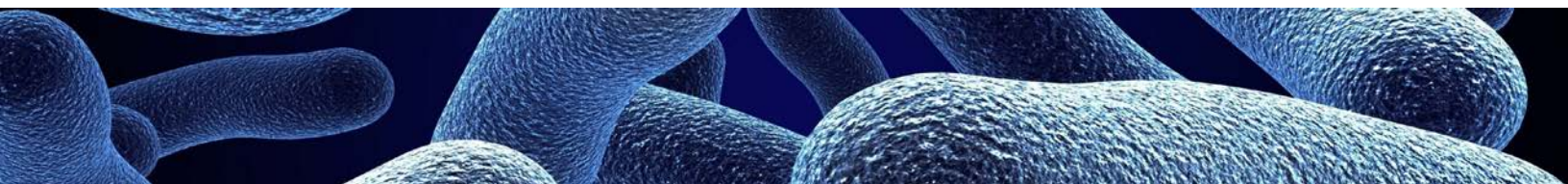
International initiatives to develop new business models, will be supported and cooperated on. This is currently happening on the 'DRIVE-AB' project, financed by the European 'Innovative Medicines Initiative', also part of the WHO Global Action Plan on Antimicrobial Resistance.

The *most important barriers in the development and registration process* will be identified, and influence will be exerted to shorten these processes. This will be done in cooperation with the Medicines Evaluation Board, the RIVM and the European Medicines Agency.

A new research program on AMR will be set up by the Netherlands Organization for Health Research and Development (ZonMw), and the Dutch participation to the European JPIAMR will continue.

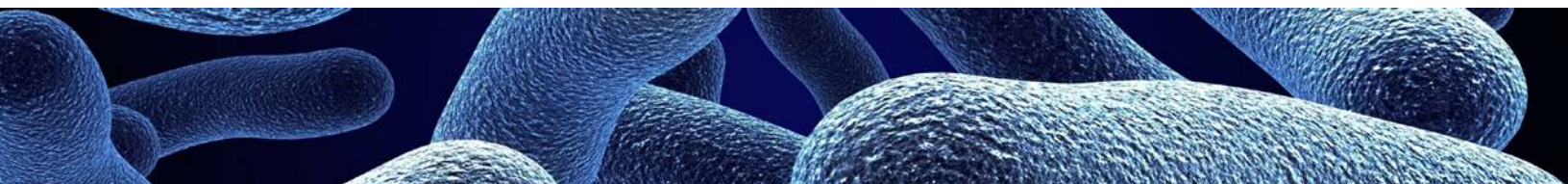
FIGURE 24: DUTCH APPROACH TOWARDS TACKLING AMR, AS DESCRIBED IN THE LETTER TO PARLIAMENT OF JUNE 2015.

In addition to stating several measures to improve and increase the development of new antibiotics, the importance of alternative treatments, infection prevention and combating the spread of the bacteria, is also acknowledged. In the letter, it is noted that some promising products have already reached the market, for example for the treatment of urinary tract infections, or acne. Therefore, it states that it is now to professionals, the National Healthcare Institute, and insurance companies to prove its value and to place them within current guidelines and insurances.



7.3.3. COOPERATION WITH THE LIFE SCIENCES & HEALTH (LSH) TOP SECTOR

Together, VWS and the LSH top sector work to strengthen the national cooperation on new antibiotics and alternatives. The Top sector LSH is one of the nine top sectors in the Netherlands, and initiates and stimulates multidisciplinary public private partnerships (PPS) to valorize innovation. It aims to boost a productive infrastructure, by attracting financial means, sharing best practices and increasing communication [167]. Its strategic ambitions for 2016-2019 are described in the Knowledge and Innovation Agenda (KIA). One of the PPPs that are ongoing or in development is AMR, in which the R&D ambition is the development of new antibiotics and its corresponding business models and laws and regulations In addition, the PPP Bacterial Vaccine Technology (Bac-Vactory) aims to develop effective vaccines for human and veterinary use.



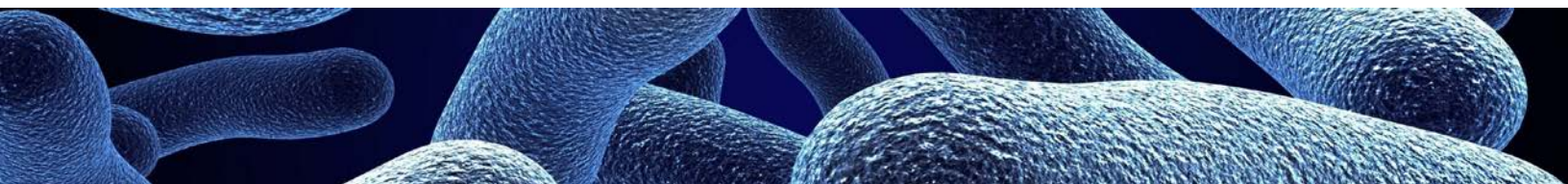
CHAPTER 8: AMR RESEARCH FUNDING POLICY

8.1. INTRODUCTION

Several initiatives have been implemented which target AMR innovation. In general, the AMR problem has a high priority on both a global, European and Dutch national level and a plethora of measures exist. As mentioned, a report by the London School of Economics' health research centre (LSE Health), commissioned by the Dutch Ministry of VWS has identified two main incentives for innovation of novel antibiotics, alternative therapies, and diagnostics[57]. First, the Netherlands Organization for Health Research and Development (ZonMw) offers direct project funding, targeting at basic research and preclinical research. Second, the Netherlands Centre for One Health (NCOH) creates research collaborations. In the letter to Parliament of June 24th 2015, innovation is seen as an essential prerequisite in order to combat AMR effectively. Therefore, a budget of €16 million was provided for AMR research. The decisions on which specific research proposals receive funding and which do not, does not lie with the government. ZonMw, an external organization, is commissioned to set up a research program, which guides solutions and research[168]. The virtual institution NCOH on the other hand, is a partnership between several universities and hospitals, which aims to set up an integrated approach to tackle the risk of infectious diseases. One of its research themes is Antimicrobial Resistance. Here, the method with which both initiatives aim to stimulate AMR innovation will be explained in more detail.

8.2. THE NETHERLANDS ORGANIZATION FOR HEALTH RESEARCH AND DEVELOPMENT (ZONMW)

Following the letter to Parliament of 2015, ZonMw was commissioned to set up a new research program on AMR. ZonMw is the Dutch national organization for health research and healthcare innovation[168]. It funds and promotes research, development and implementation. Its main commissioning bodies are the Ministry of Health, Welfare and Sport (VWS) and the Netherlands Organization for Scientific Research (NWO). To help control AMR and to foster the development of new antimicrobials, ZonMw has already set up the research program 'Priority Medicines Antimicrobial Resistance', which will fund research (with a budget of €14.76 million) over a period of 9 years (2009-2018)[169]. This program was focused on the development of new drugs. However, in the opinion of VWS, it was not realistic to expect all the solutions from one direction in a problem as complex as AMR. In addition, the amount of variants that can be produced based on our current antibiotics is running low, and it is difficult to predict the chance of obtaining new antibiotics. Therefore, a second research program was set up to have a broader approach [Appendix II.6].



The Antibiotic Resistance Program

The new research program, the Antibiotic Resistance (ABR) Program, will fund applied and basic/fundamental research as well as implementation projects over a period of six years (2016-2022; with a budget of €16 million). The process of determining the content (research areas, criteria, etc.) of the research program follows several steps [Appendix II.2](Figure 25).

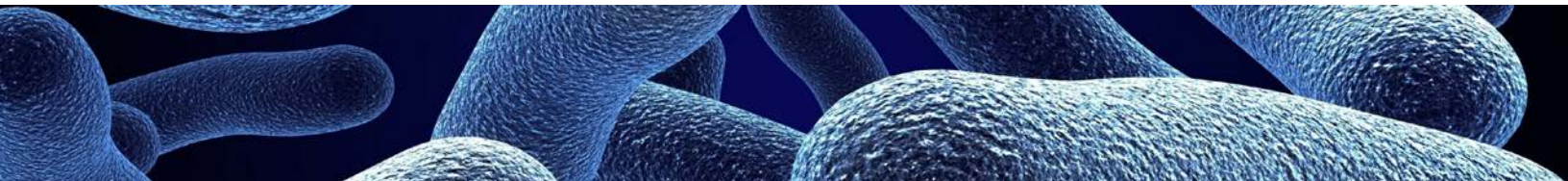
FIGURE 25: THE PROCESS OF CREATING THE ANTIBIOTIC RESISTANCE RESEARCH PROGRAM.

First, a programming study is conducted which leads to a knowledge agenda (the knowledge agenda AMR). In general, the programming study aims to assess the following matters:

- The current state of knowledge.
- The current developments (in for example political involvement).
- The possibilities for the Netherlands (relative to for example Europe).

This is achieved via a desk study of other relevant policy documents, from for example the WHO and the JPIAMR, surveys with previous project leaders and interviews with various stakeholders. The findings led to six research themes, which have been reviewed in a meeting with experts (from various research fields). Specific research questions for these themes were determined in a meeting with 60 stakeholders. Here, the role of VWS is to assess this process, whether for example enough feedback from various stakeholders has been obtained [Appendix II.6].

Next, ZonMw was formally commissioned to create the research program ABR. This was based on the knowledge agenda AMR, the strategic research agenda (SRA) of the JPIAMR, and the letter to Parliament of June 2015. VWS stressed the importance of international alignment of research and the need for outcomes that support policy making in the next five years [170].



On the one hand is this notion aimed to create awareness among researchers on the applicability of their research. On the other hand, if for example a new target for antibiotic activity in a bacterium is discovered, many stages still need to be crossed in order to produce a new antibiotic (a process that takes ± 15 years). In order to advance these developments, European programs and international cooperation are of importance [Appendix II.2].

Four research themes were identified:

1. Mechanisms for inducing and transmitting antibacterial resistance.
2. Appropriate diagnostics.
3. Mechanisms and targets for new antibiotics and alternatives to antibiotics.
4. Optimizing antimicrobial therapy: dosage and use.

After approval by VWS, ZonMw was formally commissioned to execute the research program ABR. It was noted that attention had to be given to innovation, public-private cooperation and identification of and solutions to barriers for the implementation of innovations and new diagnostics. These criteria were taken into account upon drafting the first call, together with the criteria that were set by ZonMw. One of which is the access and sharing of data obtained in the funded research.

The first call, with a budget of €4.6 million has been set out, and received 88 project ideas. The other two calls will follow in 2017 and in 2018. The members of the project committee will test these for their relevance and broadly on research quality. Approximately 20 research groups will be asked for a more extensive research proposal. These proposals are reviewed by two or more independent researchers, without a conflict of interest, from different fields of expertise [Appendix II.2]. About 10 research groups are expected to be granted funding [171].

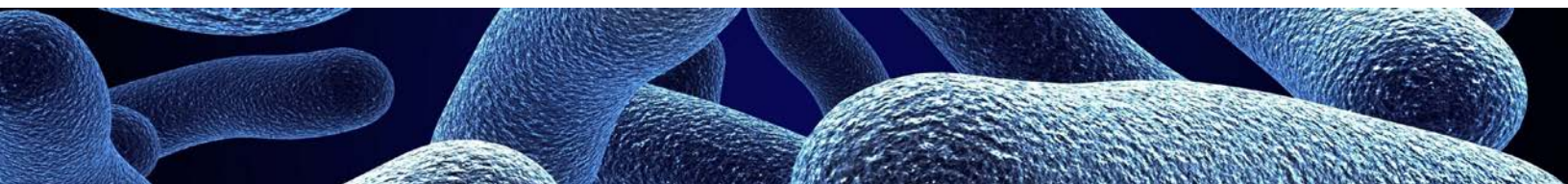
The program committee

The program committee plays a central role in this process. The committee is responsible for formulating and executing the program. The board of ZonMw assigns a diverse program committee based on their personal capacity. Criteria included the knowledge of, expertise with, and affinity for the field of ABR, and knowledge and affinity for research.
To prevent any unfair advantages, a code of conducts has been set up, and any (hints of) conflicts of interest are banned [170].

In general, a program committee at ZonMw consists out of 6-8 members, but because of the broad field of AMR, and the relatively small number of AMR scientists in the Netherlands, the AMR program committee consists out of 18 members. Observers from VWS and the ministry of Economic Affairs (EZ) will be invited to the committee meetings.

Marnix Medema, assistant professor at Wageningen University, on the sharing of data:

"We find it important that the software that we develop is open source, freely accessible. We conduct our research with public money, therefore is should be accessible and usable by the public as well."



8.3. THE NETHERLANDS CENTRE FOR ONE HEALTH (NCOH)

The Netherlands Centre for One Health was launched on the 4th of February 2016 and is a virtual science-driven institution, which creates partnerships between academic research institutions[13]. It aims to tackle the risk of infectious diseases, in a 'One Health' manner. It focuses on four research themes, one of which being 'Tackling Antimicrobial Resistance'. With the NCOH, the different partners aim to develop better research proposals in an interdisciplinary manner (and thus also increasing their chance for funding), something that is more difficult for a single research institution.

In their AMR research theme, the goal is to decrease the morbidity and mortality of antibiotic-resistant bacterial infections in humans. This problem is divided into a problem chain existing out of three problems, with different solution sets to tackle this problem (Figure 26).

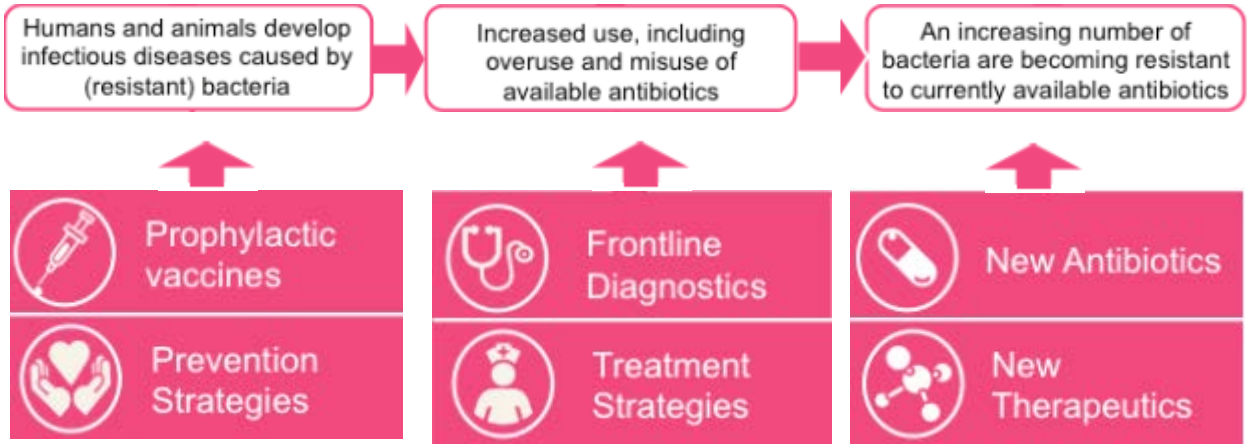
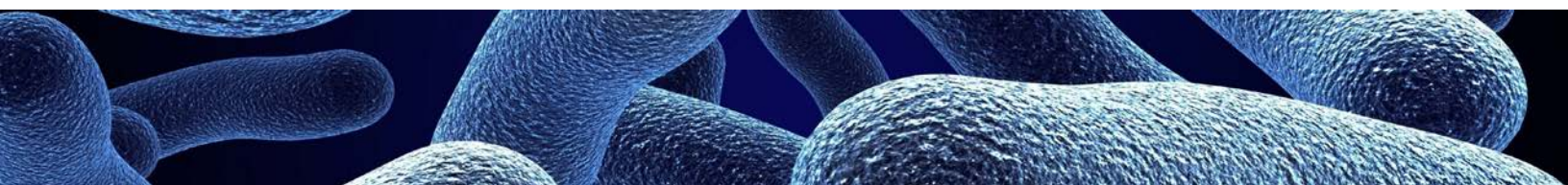


FIGURE 26: PROBLEM CHAIN AND CORRESPONDING SOLUTION SETS FROM THE NCOH. ADAPTED FROM [13]



CHAPTER 9: TOWARDS A NEW ANTIBIOTIC

9.1. THE VALUE CHAIN OF ANTIBIOTIC DEVELOPMENT

In support of the G7's Global Union for Antibiotics Research and Development (GUARD) Initiative, an advisory consortium was commissioned by the German Federal Ministry of Health to examine the key barriers of antibiotic development [5]. To this end, they first analyzed the development and marketing of an antibiotic. Five successive phases along the development and commercialization value chain were identified (Figure 27):

1. Basic research
2. Preclinical development
3. Clinical development
4. Market approval
5. Commercialization

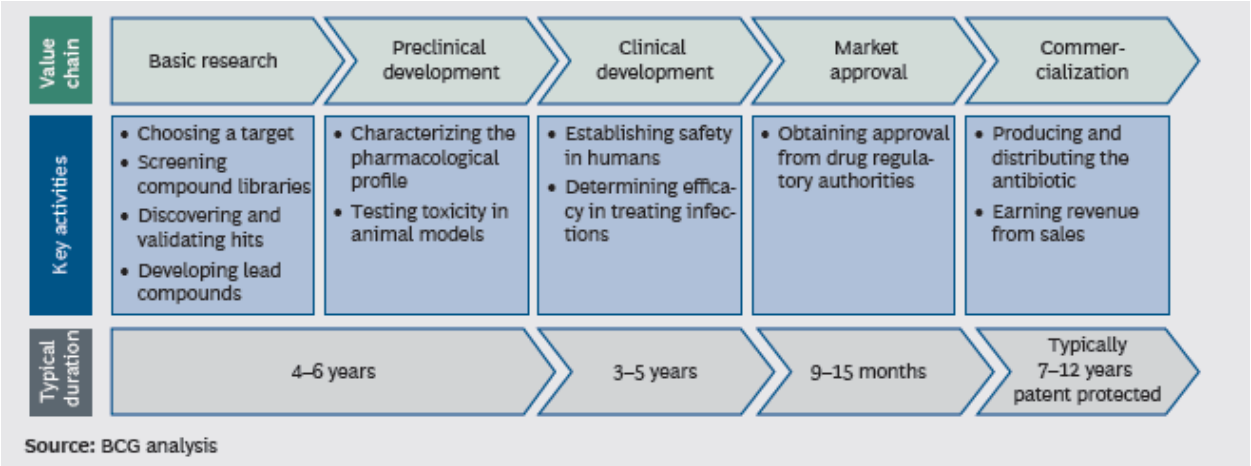
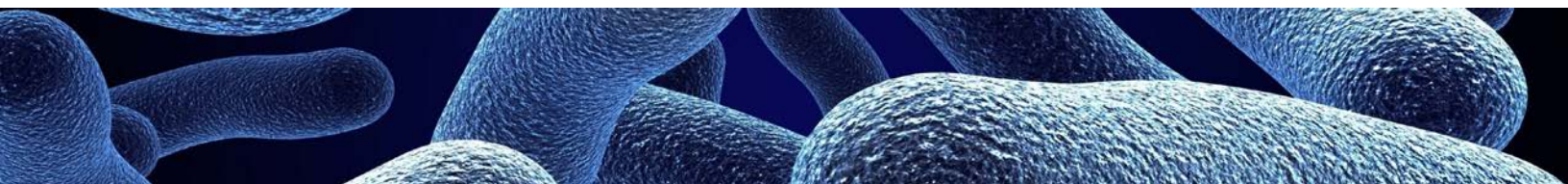


FIGURE 27: OVERVIEW OF THE ANTIBIOTICS VALUE CHAIN. ADAPTED FROM [5].

Private and public players

Although so far in this report public basic research mainly has been considered (in for example knowledge institutions), this is done by private players as well, such as large pharmaceutical companies. However, there has been a reduced investment by them in basic research, due to the low commercial attractiveness of antibiotics, described below. The number has shrunk from around 20 pharmaceutical companies in the 1990s, to five of the top 50 remaining active companies[5].

After basic research, promising scientific ideas have to be translated into clinical successes, via preclinical development. The pharmacological profile is characterized, and toxicity is tested in animal models, activities done both by knowledge institutions and by pharmaceutical companies. This phase is also called the 'valley of death', due to the lack of ideas making it into the clinical phase.

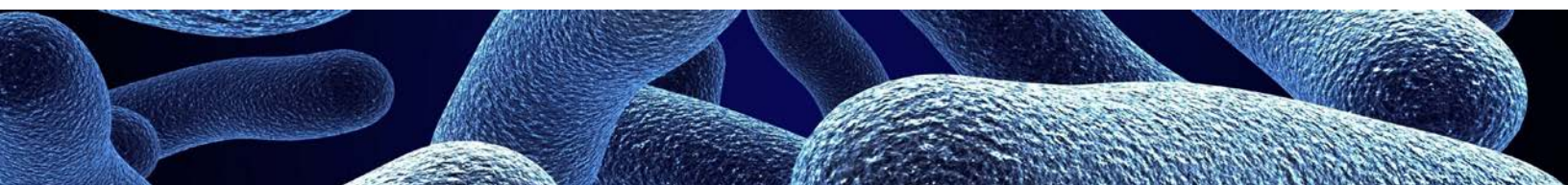


Next, during clinical development, safety in humans and the efficacy of antibiotics is tested. Pharmaceutical companies and biotech companies are able to drive this (expensive) phase. After market approval by drug regulatory authorities, the antibiotic is commercialized [5]. Other players within this value chain include for example investment companies or network organizations. An example of a network organization in the Netherlands, which focuses on antibiotic development, is Immuno Valley. They have set up a research program aiming at the development of alternatives to antibiotics for animals, by setting up partnerships between knowledge institutions and businesses, or between businesses and businesses.

9.2. MAIN BARRIERS

In the GUARD report, two major challenges were identified along the antibiotic value chain: low commercial attractiveness of antibiotics and a lack of promising results in basic research.

As can be seen, in figure 27, companies generally need to make investment decisions at least 15 years before an antibiotic comes to the market. In addition, the failure rate of research into new antibiotics is high: only between 1.5% and 3.5% of drug compounds are successful in their path from early exploration to market approval [172]. Although this is true for all types of drugs, the unpredictability of the health need is higher in the case of antibiotics. If resistance remains absent, the old antibiotics can work just as good as new antibiotics. The amount of people needing the new antibiotic is thus limited to the subset of patients that is infected with resistant bacteria. In addition, mechanisms and patterns of resistance can change rapidly and unpredictable, it is difficult to determine the health need so many years ahead [172]. Moreover, as resistance generally occurs in response to the use of a new antibiotic, these will be reserved for last-line treatment. These factors all contribute to the low commercial attractiveness of antibiotic development. To illustrate this, the O'Neill commission has calculated that profits from antibiotic research currently would only be achieved after 23 years (Figure 28). High commercial attractiveness on the other hand would lead to high activity, potentially compensating other challenges, such as those in basic research [5].



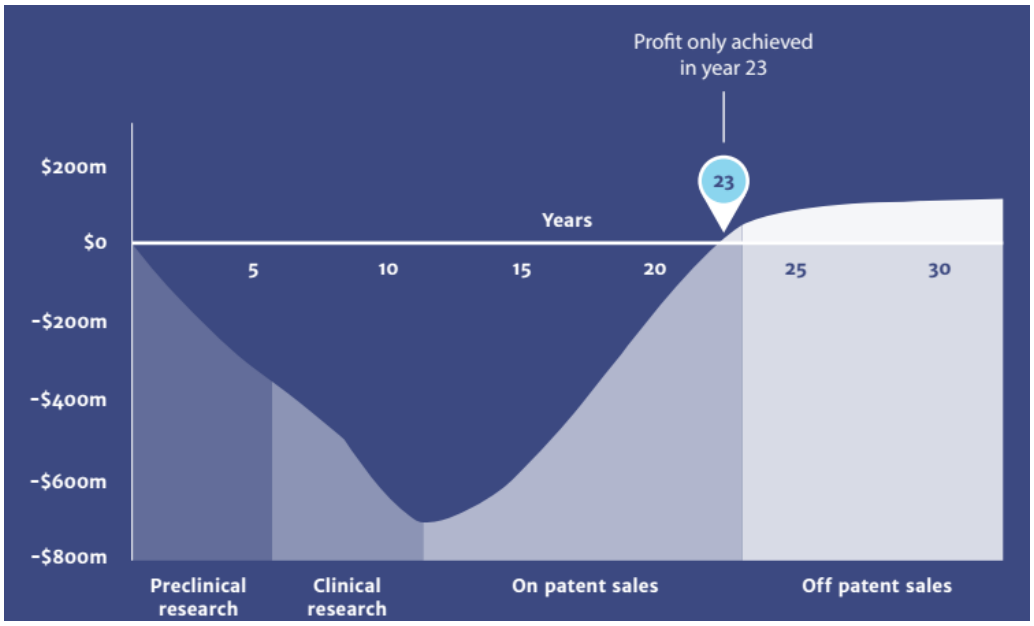
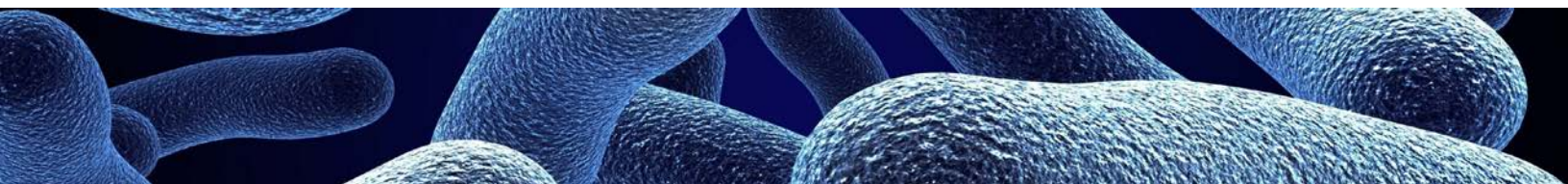


FIGURE 28: CUMULATIVE PROFITS OF ANTIBIOTIC RESEARCH WOULD ONLY BE ACHIEVED IN YEAR 23, ILLUSTRATING THE LOW COMMERCIAL ATTRACTIVENESS OF THESE TYPES OF DRUGS. ADAPTED FROM [232].

The reduced investments into basic research by pharmaceutical companies have not been fully compensated by academic institutions. This has led to a 'brain drain' of researchers. It has been estimated by multiple experts that the number of specialized antibiotic researchers has declined to only 250-500 individuals worldwide. Together with this loss of expertise, serious scientific challenges are present, increasing the problems in basic research [5]. Thus, both scientific and economic barriers exist when it comes to antibiotic research, affecting multiple stakeholders in the antibiotic innovation pipeline.



CHAPTER 10 – DIFFERENT POINTS OF VIEW

10.1. INTRODUCTION

One of the stakeholders in the AMR innovation process is the government, which could aid in removing the scientific and economic barriers mentioned. They currently do so directly (on national level) mainly via the initiatives mentioned in chapter 8. In order to gain insight into the considerations that can be made regarding their role in AMR (synbio) innovation, several interviews and a questionnaire were held. To this end, different stakeholders at various points in the antibiotic innovation pipeline were determined and they were asked on their viewpoints on this matter. These stakeholders are all involved in different processes of this pipeline, and thus have point of views relating to this involvement. A full list of interview participants can be found in Appendix II.1.

As mentioned, both introductory/exploratory interviews were held, as well as semi-structured interviews. In addition, one interview was held for critical reflection. The answers that were given in these interviews were categorized into the following categories and can be found in Table 5:

- The most prominent solution direction that was seen for AMR in general.
- The main barriers in the current development of new antibiotics/alternatives/diagnostics.
- Who should make the next move in order to help these developments further.

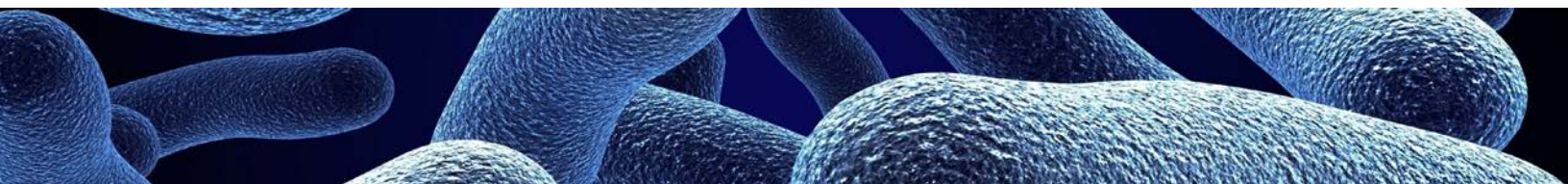
Not every participant in the interview was familiar with synbio in such a way as to determine the barriers and chances for synbio in AMR. In addition, while some answers were mostly focused on synbio in AMR in a broad sense, some were more specific (mainly from scientists).

The questionnaire was held among mostly AMR scientists and aimed to gather their opinion of on the viability of the current developments of new antibiotics and alternatives to antibiotics. They were also asked what was needed in order to help the developments further, and whose next move would be most important in order to do so. The questionnaire, and a more extensive report on the results, can be found in Appendix IV.

10.2 RESULTS

In the table below (Table 5), the results of the interviews are shown. As mentioned, not all stakeholders had enough specialized knowledge on the developments within synbio that contribute to tackling AMR in order to provide these answers. The results in part one could contribute to increasing their knowledge on these developments.

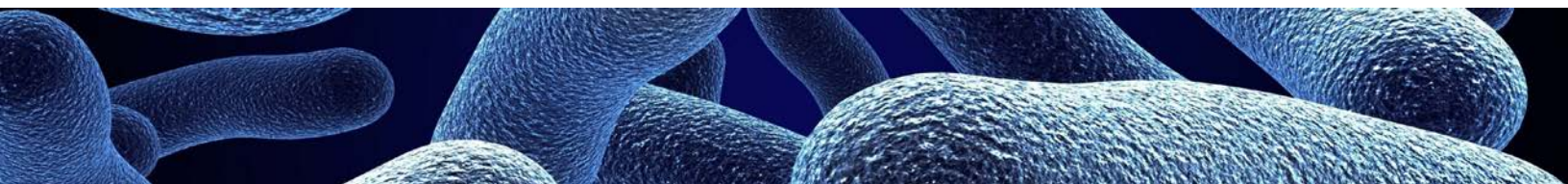
Therefore, only the stakeholders that could provide an answer regarding the chances and barriers of synbio are shown. These stakeholders often provided multiple answers (Table 6).



10.2.1. RESULTS REGARDING THE ROLE OF THE GOVERNMENT IN AMR INNOVATION

Stakeholder	Type of interview	Process involvement	Solution direction	Main barriers	Whose next move?
Prof. synthetic biology.	Introductory	Research	-	Economic barriers.	-
Head of medical microbiology at a hospital (1)	Introductory	Use of the product	New antibiotics with a new working mechanism + infection prevention and surveillance	Economic barriers	Government
Policy maker VWS (1)	Introductory	Policy development	Infection prevention and surveillance	Economic barriers	-
Ass. Prof. Molecular Microbiology	Introductory	Research	-	-	Government
Ass. Prof. Bioinformatics	Introductory	Research	Not one solution	Economic barriers	
Head of AMR funding program	Introductory	Research funding	-	-	-
Investment professional	Semi-structured	Research – Development & production	More recognition of the problem	Economic barriers	Government
Policy maker VWS (2)	Semi-structured	Policy development	Infection prevention and surveillance	Mostly scientific, to a lesser degree economic barriers	Industry and scientists
Network organization and service office managing director	Semi-structured	Research – Development & production	-	Both scientific and economic barriers, intertwined	Various
Head of medical microbiology at a hospital (2)	Semi-structured	Use of the product	Infection prevention and surveillance	Economic barriers	Government and knowledge institutions

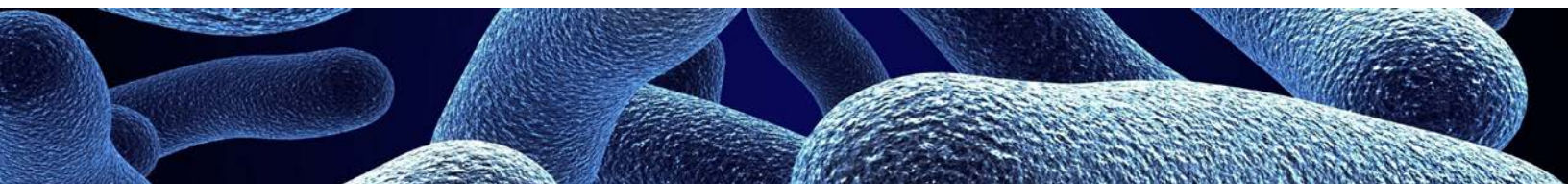
TABLE 5: RESULTS OF THE INTERVIEWS, ANSWERS ARE CATEGORIZED INTO THREE GROUPS.



10.2.2. RESULTS REGARDING THE CHANCES AND BARRIERS OF SYNBIO (IN AMR).

Stakeholder	Type of interview	Process involvement	Chances	Barriers
Prof. synthetic biology	Introductory	Research	Potential in AMR, but not necessarily biggest spin-off of synbio.	Synbio research takes time.
			Optimalisation of production pathways with synbio.	
			No resistance mechanisms yet against new-to-nature molecules.	
Ass. Prof. Molecular Microbiology	Introductory	Research	Endolysins	Laws- and regulations
				Bacteriophages: no complete alternative for antibiotics; resistance development
Ass. Prof. Bioinformatics	Introductory	Research	Potential, synbio will be a game-changer in the next 20 years	
			Big impact on the discovery of new natural products	
			Favorable public opinion in general	Public opinion on GMOs in plants not as favorable
Investment professional	Semi-structured	Research – Development & Production	Not the most obvious research area, but a lot has gone right lately	Perhaps public acceptance, although GMO acceptance is better
Network organization and service office managing director	Semi-structured	Research – Development & Production	General chances for synbio in AMR	Laws- and regulations
				High costs
Head of medical microbiology at a hospital	Semi-structured	Use of the product	Chances for synbio in diagnostics	Development of resistance always occurs (also with new molecules)

TABLE 6: RESULTS OF THE INTERVIEWS REGARDING THE CHANCES AND BARRIERS FOR SYNBIO (IN AMR).



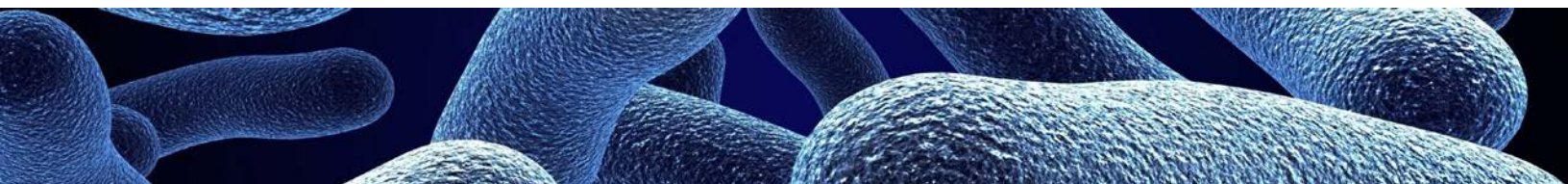
10.2.3. RESULTS OF THE QUESTIONNAIRE

For a more extensive report on the questionnaire results and a description of the background of the participants of the questionnaire, see Appendix IV.2. Here, the most important results are given (Table 7).

Question	Type of question	Result (most given answer)	Result (least given answer)
On what would you spend a hypothetical large sum of money, towards the development of new antibiotics/alternatives?	Open	Mining/screening for new antibiotics in nature	-
What development has the highest potential?[1]	Closed	Modifications to existing natural compounds	Lysins
What is needed in order to help these developments further?	Closed	Scientific interventions (scientific education, infrastructure for research, more scientific cooperation)	-
Whose next move is most important?	Closed	Government and knowledge institutions	-

TABLE 7: RESULTS OF THE QUESTIONNAIRE.

[1] *Potential has been defined as 'being able to reach the market the fastest'.*



10.3. DISCUSSION

10.3.1. THE PROBLEM AND ITS SOLUTIONS

As mentioned before, AMR is a complex and multi-faceted problem that requires a similar approach. Therefore, it is not realistic to expect a single solution to be the answer to this problem. It seems however, that in the Netherlands, infection prevention and surveillance (also of resistant bacteria from 'abroad') are generally perceived to be of main importance. As one medical professional said: "It is not enough to create new antibiotics, as we did in the '60s and '70s. The use of an antibiotic should be the exception, and resistance needs to be prevented as much as possible". This view is also shared by policymakers of VWS. Although another medical professional agreed with this viewpoint, he also underscored the critical importance of the development new antibiotics, with new working mechanisms. From his perspective, it is not necessary to know were these antibiotics would come from, as long as they are safe and efficient. It was also stated by a medical professional that only antibiotics for the most urgent and important bacteria are needed. A reason according to which it is thought that broad screening for antibiotics would perhaps not be very effective. In addition, it is also preferred that the antibiotics that are used are targeted ('smart antibiotics' and directed drug delivery). In order to achieve this, he thinks appropriate diagnostics will be necessary. "No doctor is nowadays able to properly diagnose an infection with antibiotic resistant bacteria without the appropriate microbiological diagnostics".

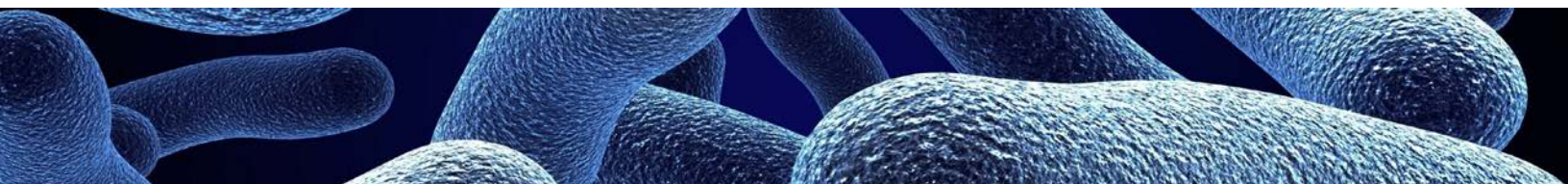
Conclusion

From a policy point of view and a medical point of view, the development of new antibiotics and alternatives is not necessarily the most important solution towards the tackling of AMR. Infection prevention and surveillance are seen as most important, although there remains some disagreement. However, in the development of new antibiotics, the focus should lie on their functioning against the most important and urgent bacteria, in a targeted manner. In order to achieve this, appropriate diagnostics would be necessary.

10.3.2. RESEARCH FUNDING

The distribution of funding provided by the government is currently executed by ZonMw. Whereas the previous research program AMR focused mainly on the development of new antibiotics, the current research themes are broader. These themes correspond with the view that the development of new antibiotics alone will not solve the problem. As mentioned before, these themes are based on several interviews, reports and surveys. A wish from VWS is that the outcomes of this research will contribute to choices by policy makers in the tackling of AMR.

This should challenge research groups to already think about the applicability of their research. The decisions on the actual division of this funding is done within the program committee, with the help of (foreign) experts. A process that is seen by one scientist as suitable, although he would think of the expert panel to exist also out of companies.



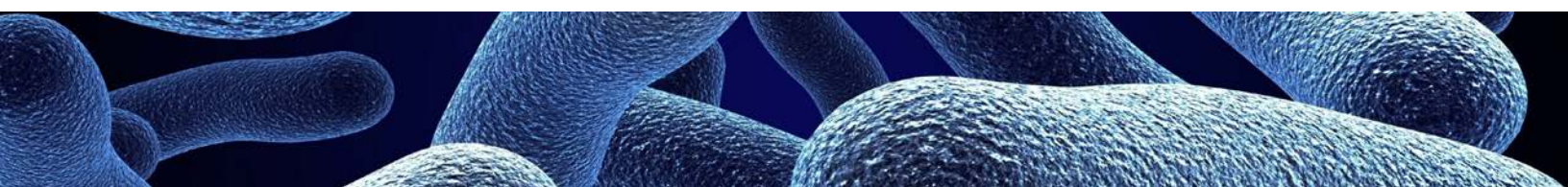
On the other hand, in the interview that aided in critical reflection of the answers given, it was stated that this managing of scientific funding has carried on too far and an institutionalized distrust towards scientists has arisen. Because of the long procedures and external experts, it was thought that the tendency exists by the funding organizations to abstain from taking responsibility. In addition, the high procedural costs could also be spend on more research. The interviewee therefore liked to see a bigger mandate towards knowledge institutions, leaving the division of funding op to them.

One medical professional notes here that in his opinion, the current research programs are very focused on academia, but co-creativity is not necessarily confined to research centers. Participation and involvement in decision making by the people at the end of the innovation chain is also important. The importance of research has to be seen by everyone (including citizens and patient organizations), which could also lead to more out-of-the-box ideas.

Guiding research

Guiding research more (in addition to the existing research themes) towards a specific direction of research at this point can be difficult or unwanted. This is exemplified by the discord in the answers of the questionnaire participants. When a group of 60 AMR stakeholders (mostly scientists) was asked which developments have the most potential to provide us with new antibiotics or alternatives, there was little agreement. However, when openly asked 13 of 60 questionnaire participants would fund the mining/screening for new antibiotics in nature. When given a set of answers to create a top 3 out of, 33 of 60 participants would choose to create modifications to existing compounds as the development that would lead to a product closest to the market. However, this might be because potential was defined as 'being closest to the market'. Mainly from a scientific point of view, and based on critical reflection, it is also thought that the current funding criteria already guide research too much, and guiding the results of research is not possible. In addition, concerns have been raised over the amount of creativity researchers still have when research criteria are being set too strictly.

On the other hand, specific wishes exist in healthcare settings when it comes to research outcomes. As mentioned by one medical professional, healthcare professionals would like to have antibiotics towards the most important and urgent bacteria that function in a personalized manner, together with appropriate diagnostics. However, while one scientist understands these wishes from a medical professionals' point of view, he sees difficulties in fulfilling these wishes. He states that antibiotic research and development require a lot of time, until a product is ready to be used (10-15 years). This might cause that although research is matching clinical needs at that time, it might not be any more when research has finished. 10-15 years later, different multi-drug resistant pathogens might have to be targeted than those right now. He therefore thinks current research should focus more creating a multiple different therapeutics, with different working mechanisms, from different fields of research.



The role of the government

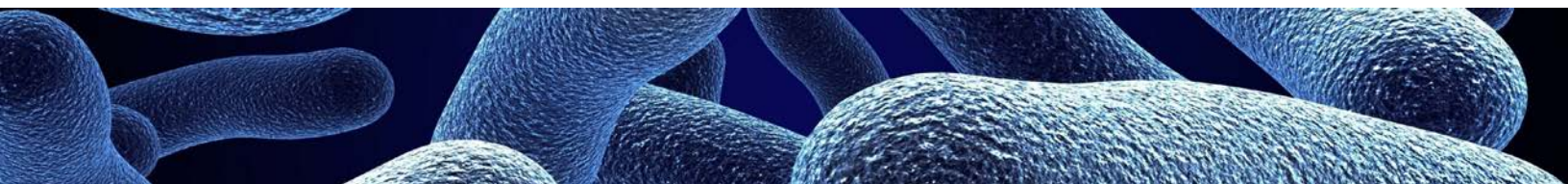
Should research indeed be more guided towards these demands? And if deciding upon matching healthcare demand and ongoing research, whose role should it then be? Public health is generally the responsibility of the government. In addition, antibiotic effectiveness is an economic good that has some characteristics of a public good. Public goods are non-rivaled and nonexclusive in consumption. Public goods are usually either provided by a governmental agency or by some type of collective organization [173]. Therefore, is can be argued that some role of the government in this process would be required. From the perspective of a network organization, it was therefore suggested to think of two separate lines of AMR research: explorative (basic) research in which the government does not play a role, and market- and healthcare- guided research in which the government can play a facilitating role.

Currently, there is no clear guidance by the government to stimulate particular developments to would lead to new antibiotics, alternatives or diagnostics. The government finances AMR research, and guides the main themes of the AMR research program of ZonMw. However, the responsibility of dividing this research funding lies with ZonMw. From a policy perspective, it is generally difficult to make a well-substantiated decision about the things that are needed the most. Policy makers generally lack the specialized knowledge to select the development with the best potential. In addition, policy makers might have a less comprehensive view of the end of the innovation chain. An interviewee that critically reflected on this matter stated that this is not necessarily a problem, as long as there is enough trust in the scientists that conduct the research. As mentioned, a fear also exists that too much guidance from the government will come at the expense of creativity in research. The best innovations have originated ‘coincidentally’, or as a byproduct of research.
However, according to 60 stakeholders involved in AMR, mostly scientists involved in research (n=56), the next move of the government is indeed very important. A viewpoint that is also shared by other interview participants throughout the innovation pipeline. Could the government then play a different role in the innovation pipeline instead of guiding research?

Conclusion

Specific wishes and needs at the end of the antibiotic pipeline may require research to be more directed towards those specific demands. It can be argued that, because AMR is often seen as a public health problem, and antibiotic effectiveness has some characteristics of a public good, a role for the government is laid out. However, the steering or guiding of research is generally perceived to come at the cost of creativity, something that many innovations we currently have is based on. In addition, it is thought that the results of research cannot be steered or guided at all. Knowledge on current developments, healthcare demands (patient and medical professional) and market demands would be necessary to guide research.

Currently, policy makers often lack the specialized knowledge to decide upon the potential of a certain development. Therefore, guiding or steering research (and its results) towards the specific demands might not be the most suitable role for the government.



10.3.3. FROM RESEARCH TO PRODUCT

Together with a role for the government, AMR scientists see a large role for knowledge institutions to help the developments leading to new products further. Interventions focusing on academia and research are therefore valued the most in the questionnaire. Scientific education, infrastructure for research and more scientific cooperation are seen as most important, although the importance of interventions further in the development chain is seen as well. However, most participants of the interview do not see scientific barriers as the main barrier holding back the development that could lead to new antibiotic products or diagnostics.

Although scientists are generally focused on publishing, something that is also required by the university, they are not as naive as generally thought when it comes to bringing their research to practice, according to a scientist. Or, as one medical professional puts it, universities, together with the government, have the responsibility to bring knowledge into the society. However, he also states that knowledge institutions generally are limited in their means to advance this knowledge further. Therefore, small- or medium enterprises, or big pharma are needed to develop this knowledge into a product.

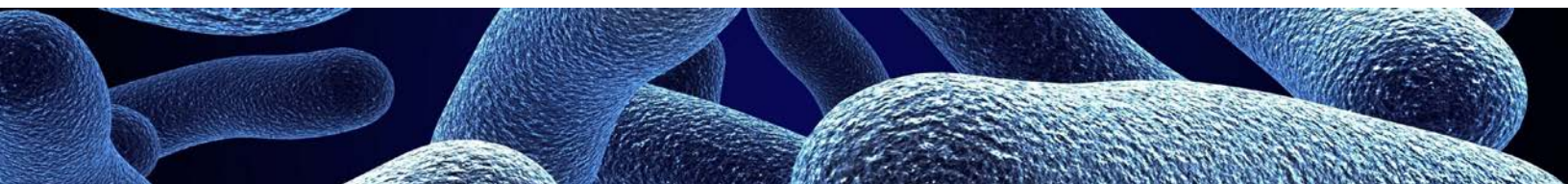
The business model of antibiotics and diagnostics

Almost all participants of the interviews at various points in the innovation chain see the business model for the development of antibiotics as the main barrier. An economic barrier is also seen as most important for the development of new diagnostics, especially by medical professionals. In the current model, diagnostics are not reimbursed; meaning doctors or nursing homes have to pay for these out of their own budget. Indicated diagnostics are therefore an expense, which creates a barrier for their use. These barriers could prevent industry or investment companies from investing in antibiotic or diagnostic research. For an investment company for example, the chances of an investment are determined by the use of that product by people. It was noted here by the interviewee from a network organization, that economic barriers cannot be seen separately from scientific barriers. “The low-hanging fruit has already been picked, and the process of antibiotic discovery is not as it used to be, it has become complex. Because of this complexity, the risks and costs have become high, creating economic barriers as well.”

Solutions and the role of the government

If mainly economic barriers are seen, antibiotic innovation should therefore maybe not depend on the market alone. This could implicate that a role of the government is needed. The participants of the interviews have suggested several roles of the government to be involved in this process. .

First, *economic incentives* can be created that can stimulate the industry to invest in antibiotic and diagnostic research and development. A comparison was made by an investment professional and a policy maker between antibiotic and orphan drugs. For both products, the amount of people that need it, and is going to use it, is small. The Orphan Drug Act, which consisted out of relatively small incentives, created a large effect. Such an approach could also be taken when it comes to the development and production of antibiotics.

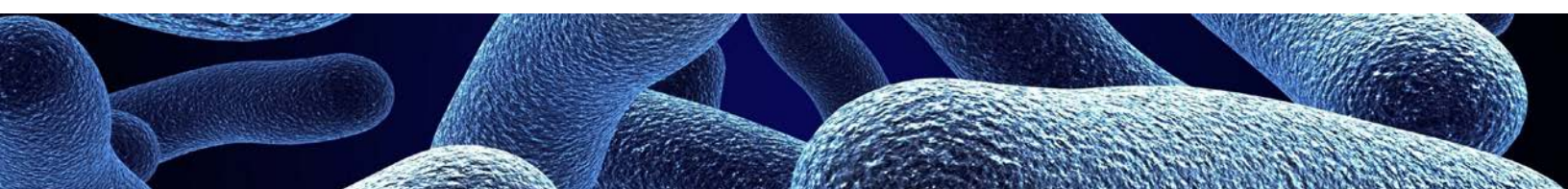


Second, one medical professional would like to see the government participate more in the *process of technology transfer*, thus stimulating the ongoing developments in an early stage. Although the industry generally steps in to fulfill this role, as mentioned, financial incentives to do so are currently lacking. In his proposal, he states one model could involve the industry on one point taking over these developments from the government, after which a certain percentage should be paid back to society. However, according to a policy maker, governments are generally reserved when it comes to an active involvement in processes like technology transfer. This is probably due to the same reasons for which they remain reserved in guiding research, deciding upon which developments to stimulate and which not. However, it could also be because of the fact that the government generally wants to safeguard its public role, thus not actively cooperating with the industry.

Third***,*** *public-private partnerships (PPPs)* could be increased, also one of the main current approaches of the government. It also recurs as one of the criteria of the AMR research program of ZonMw. From the perspective of a policy maker, these public-private partnerships should be enhanced. However, a risk of this approach is also mentioned: “When spending public money, a different moral duty exists, as compared to spending private money. In the first case, different societal problems, for example those of less-wealthy countries, are taken into account. In the latter case, there are often no societal goals, mainly financial goals. In public-private partnerships, this dividing line is more blurred, perhaps creating indistinctness about the moral duties that exist. A risk that exists then, it that private goals could be followed more often than public goals. This is a process that needs to be guarded at all times”.

Governments, or governmental agencies are therefore generally reserved in their involvement with industry. For them, it is of great importance to safeguard the public interest. According to an interviewee from a network organization, it is possible, and necessary, to create collaborations with industrial partners: “Networks will be necessary to solve complex issues such as AMR.” One method of avoiding these problems, is by instating non-profit companies that position themselves between research and business.

However, because of the gravity of the AMR problem, and the fact that it involves public health, still requires the government to have some sort of influence or responsibility. By funding these types of companies, their existence is not compromised, or left to the unpredictability of the market. In addition, the government can keep a source of knowledge (both on scientific and business developments) close.



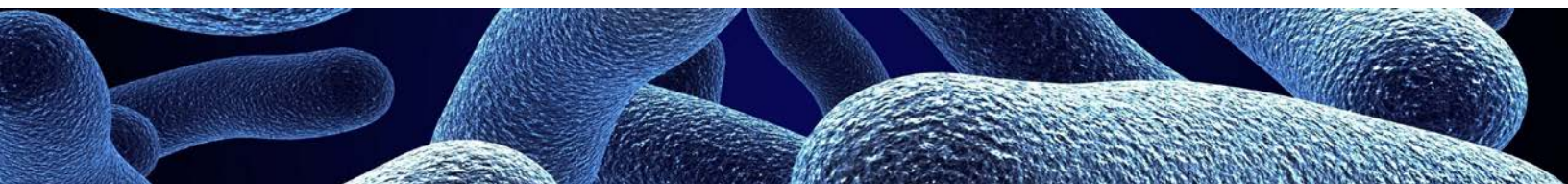
Conclusion

A big role is generally contributed to knowledge institutions when it comes to advancing AMR developments further. Scientific education, infrastructure for research and more scientific cooperation are seen as the most important interventions from a scientific perspective. However, the most important barriers are perceived to be economical, although these might be difficult to see separately from scientific barriers. This could implicate a larger role for the government, as antibiotic innovation can therefore not depend on the market alone. Several solutions towards removing these barriers have been suggested by the participants of the interviews:

- Creating economic incentives
- Facilitating technology transfer
- Creating public-private partnerships

The government can play a role in all of these solutions, all in a different manner. While the creation of economic incentives is seen as a small intervention with possible big results, facilitating technology transfer actively might pose more difficulties, for example in the selection of developments with the most potential.

One other way the government could be involved in creating solutions towards economic barriers, is the facilitating of PPPs. To avoid the risk of not safeguarding public interest, the government could facilitate external companies to create these collaborations. This way, the government could also easily stay up to date on both scientific and business AMR developments.



CHAPTER 11 – CONCLUSIONS AND RECOMMENDATIONS

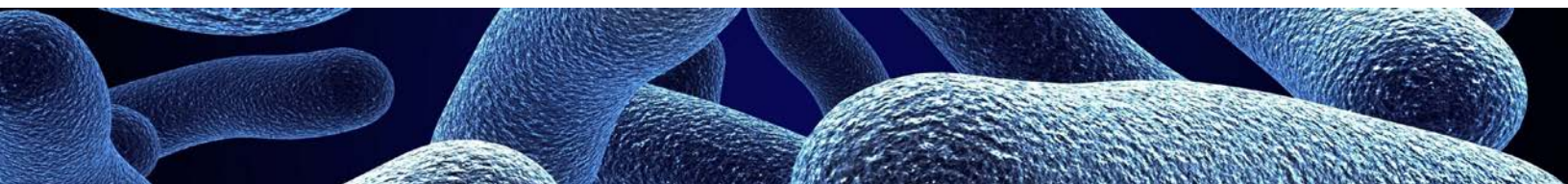
In this report, two objectives are set out to be achieved:

> *1. To create an assessment of the developments in synthetic biology that are relevant for antimicrobial resistance. This includes both new antibiotics, alternatives to antibiotics, and diagnostics.*

> *2. To map the considerations that can be made by the government on their role in (synbio) innovation in AMR.*

11.1. CONCLUSIONS OBJECTIVE 1:

Synthetic biology is a quickly emerging field of research, which not only offers new tools, but also a new way of thinking. It approaches biology with an engineering perspective, characterizing, designing and constructing novel biological systems. This transformation in thinking is not necessarily very recent, but the field has become more popular and has grown and evolved rapidly. This is due to for example the increasingly cheaper production of DNA and our growing knowledge of DNA sequences. Among the many applications of synthetic biology, is its contribution to tackling health problems, such as antimicrobial resistance. The ways it could do so, have been described extensively in this report, and are summarized in Table 8. It has to be noted that licensed and clinically approved applications are generally still far away – almost all developments mentioned in this report are still in their proof-of-principle phase. The development of novel diagnostics however, is subjected to less strict regulations than the development of novel therapeutics, which could lower their time towards a clinically approved product. Stimulation of research or the improvement of regulations (with regard to its time consuming nature) in drug approval could aid in advancing these and other developments further. Because of its wide range of applications and its speed of development, there are many expectations for synbio. Although it is said that the developments within AMR are probably not the biggest spin-off of this field (more likely the use of bacteria as factories for biofuels or platform chemicals), much is still expected for the use of synbio in drug discovery. There are many molecules, either natural or new-to-nature to be discovered, in which synbio could be of help. In the Netherlands, the specific synbio/AMR research field is small, and much more is being done towards other applications of synbio. The synbio/AMR scientists therefore see international cooperation (for example with the UK or the US) as necessary for successful research and development.



Novel antibiotics	Alternatives to antibiotics	Diagnostics
Target identification using the minimal genome approach	Engineered bacteriophages	Pathogen detection using improved bacteriophages
Screening platforms	Engineered probiotics	Paper-based diagnostics
Discovery of novel natural antibiotics by awakening 'silent' BCGs	Engineered lysins	
Creating new-to-nature molecules	Accelerate vaccine availability/creating 'smart' vaccines	

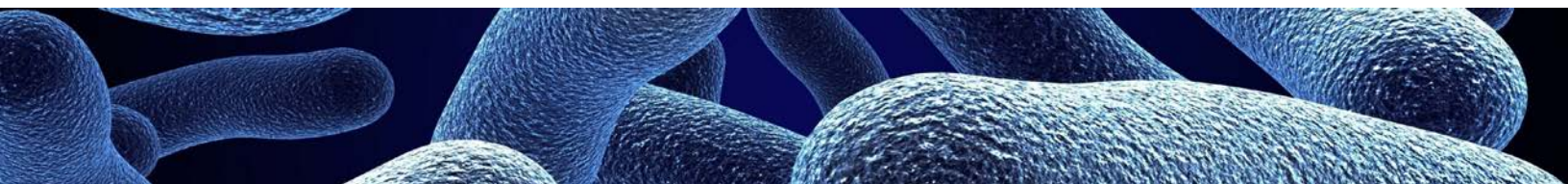
TABLE 8: SUMMARY OF THE SYNBIO DEVELOPMENTS THAT COULD CONTRIBUTE IN TACKLING AMR.

11.2. CONCLUSIONS OBJECTIVE 2:

The complexity of the field of AMR is huge – there are many aspects, processes and stakeholders to take into account. Considering the scope and available time, this report therefore only serves as a first analysis of the more comprehensive underlying data. A more detailed scrutiny of data and context, combined with additional stakeholder research would enable a more fine-tuned analysis.

To tackle such a complex problem as AMR, different approaches at once are needed. In the Netherlands, the solution directions that seem to be most important are infection prevention and surveillance. For this, appropriate diagnostics are seen as a prerequisite. This is probably due to the fact that AMR is relatively 'under control' in the Netherlands (in comparison to countries such as Greece). The need for innovation is therefore maybe less felt. However, the importance of the development of new antibiotics and alternatives to antibiotics is definitely acknowledged. Therefore, both the solution sets of the NCOH and the research themes of ZonMw are broad, exemplifying the different approaches that are needed.

The stakeholders in AMR innovation are relatively unfamiliar with the developments in synbio that can contribute to tackling AMR. However, as this report shows, it offers a wide range of applications, contributing to the development of both new antibiotics and alternatives, and diagnostics. In addition, it can aid in answering several fundamental questions. This report can therefore contribute to the knowledge of these stakeholders on synbio/AMR innovation.



Roles for the Dutch government

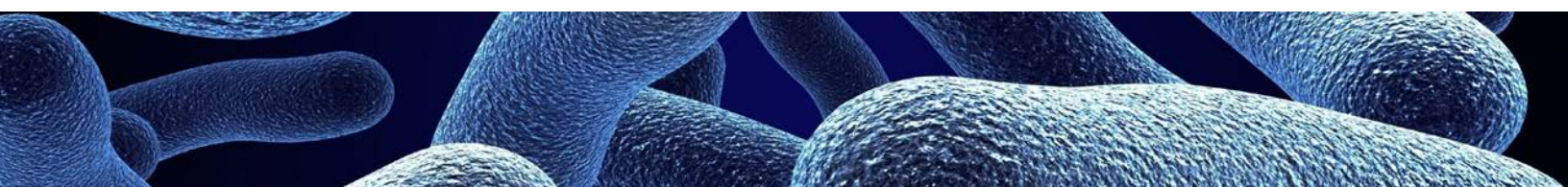
This report has focused on the part of the Dutch government that plays a role in AMR innovation, mainly the ministry of VWS. To stimulate AMR innovation, which includes AMR innovation in synbio, the Dutch government can pursue different roles. The roles that have been mentioned in this report can be divided into two processes of the antibiotic innovation pipeline: a role in research, and a role in the process from research to product.

1. Role in research: From a medical professional point of view, it is important to create better cooperation between scientists and end-users, especially in a public health problem with a large public interest such as AMR. A specific health need exists, that not necessarily aligns optimally with current research. This raises questions on the matching of health demand and current research developments, something that does not seem to be actively pursued on the moment. This might be because of practical issues - research focusing on current healthcare demand will only provide products in 10-15 years, when healthcare demand might be different. In addition, research results are generally seen as difficult, if not impossible, to guide. In any way, better interaction and sharing of knowledge between scientists and end-users could aid in the mismatch that seems to exist between expectations and the actual research outcomes that can be achieved.

2. Role in the process from research to product: Scientific barriers however, were generally not seen by stakeholders as most important. Throughout the antibiotic innovation pipeline, economic barriers preventing the further development of research outcomes, were seen as most important. The business model of antibiotics is not optimal, but a need for novel therapeutics and diagnostics exists. This indicates that the market alone will probably not be able to fulfill these needs, and an initiating role for the government exists. Several solutions towards removing these economic barriers have been suggested from various stakeholders in the antibiotic innovation pipeline: creating economic incentives, facilitating technology transfer, or creating public-private partnerships.

While the creation of economic incentives is seen as a small intervention with possible big results, facilitating technology transfer actively might pose more difficulties, for example in the selection of developments with the most potential. A problem that is also seen in guiding research. In addition, the Dutch government could also be involved in facilitating PPPs (both financially and logistically). Currently the establishment of PPPs towards the development of antibiotics/alternatives/diagnostics for human use, is mainly left to other stakeholders.

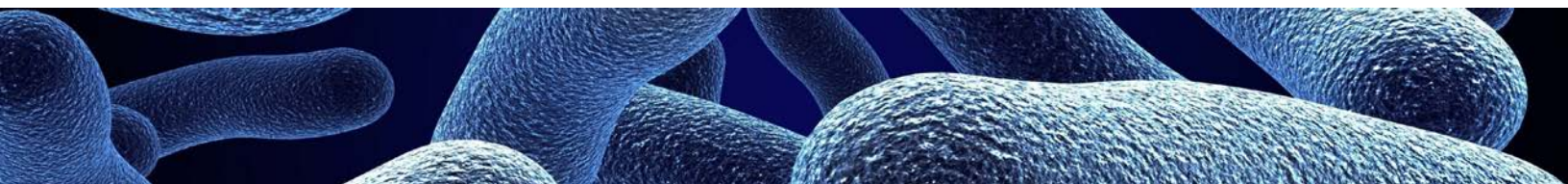
A risk that exists for an active role of the Dutch government here, is not safeguarding public interest, by creating a direct cooperation between the government and businesses. In addition, concerns have been raised about the ability of the government (a public party) in pursuing its public goals, while partnering with private parties with other, private goals.



Considerations

Upon deciding on their role in stimulating AMR innovation, the ministry of Health, Welfare and Sport (VWS) have to take into account different aspects of this process. Based on the viewpoints of the different stakeholders and based on the roles that have been mentioned above, different considerations have been extracted. Although many more considerations can be made, the following can already be taken into account:

- Is AMR a public health problem? Can antibiotics (or their effectiveness) be perceived as a public good?
- Does the demand (new therapeutics towards the most urgent and important bacteria) need to be matched with current research and market demand? Can research outcomes be guided at all?
- If so, does the Dutch government have the knowledge to decide upon the potential of ongoing developments and research? If not, who does?
- Does the Dutch government need to pursue a more active role in facilitating scientific interventions in AMR, such as the establishment of a non-virtual research institute?
- Can the Dutch government pursue an active role in facilitating active technology transfer? And can public goals and public interests still be safeguarded when doing so?
- Should the Dutch government do more in creating (small) financial incentives?
- Does the Dutch government need to pursue a more active role in facilitating public-private partnerships? If not (for example because the public interest cannot be safeguarded in doing so), should it actively facilitate an external organization to create these PPPs?



11.3. RECOMMENDATIONS

1. There are several synbio developments that could be part of the AMR innovation pipeline and they seem to offer novel approaches in various manners. However, almost all of them are in their proof-of-principle phase and still remain far from licensed and clinically approved products. In addition, this specific field (AMR/synbio) is relatively small in the Netherlands. It is therefore recommended to:

Closely monitor these developments, not only on national level, but also on international level, in order to identify barriers (for example regulatory barriers) in further development and production and timely remove these, while safeguarding risks and considering societal and ethical issues. The RIVM, more specifically the Centre for Safety of Substances and Products (VSP), has the potential to do so, as it aligns well with their current activities in signaling new developments within modern biotechnology.

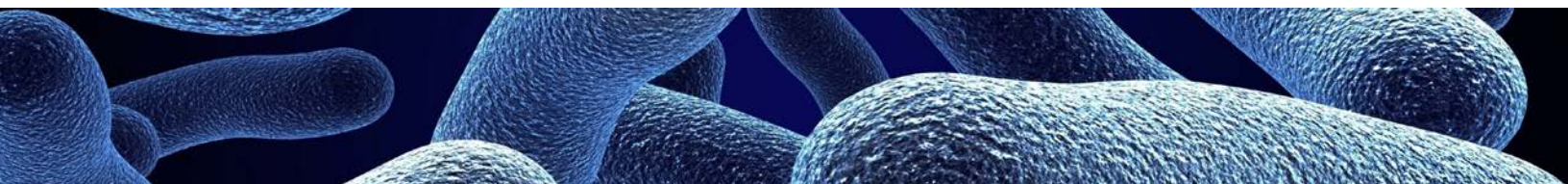
A close monitoring of these developments is necessary to gain early insight and to detect any upcoming problems. In addition, it might prove useful in removing any barriers in their innovation pipeline, such as regulatory barriers.

The knowledge obtained by the RIVM, therefore has to be shared with the Direction Medicines and Medical Technology of VWS, as they are responsible to remain up-to-date with the current AMR developments and can aid in the removal of these type of barriers. As VWS is not the main commissioner of VSP (this is the ministry of I&M), it can be beneficial to create a better cooperation with the Centre for Infectious Diseases (CIb) (whose main commissioner is VWS).

2. The Dutch government, mainly for the ministry for Health, Welfare and Sport (VWS), in stimulating AMR innovation could play a bigger role in stimulating AMR developments. Although synbio developments in AMR bare great potential, many other research areas do as well. No specific distinction can therefore at this moment be made in this report towards stimulating any particular development.

Economic barriers in the development of novel therapeutics and diagnostics seem to be most important, while networks between all stakeholders within the antibiotic innovation pipeline seem to be essential. The considerations that are mentioned in this report could aid the government upon deciding on their role in stimulating AMR innovation. However:

Additional research upon the viewpoints of various stakeholders in the antibiotic innovation pipeline is necessary to obtain a more comprehensive view on the perceived role of VWS within the stimulation of AMR developments.



Taking the aforementioned considerations into account, a personal recommendation on the role of the government has been made:

Facilitate an external network organization, financially independent from the market, which can:

- *Create a network of, and (actively) facilitate cooperation between, both private and public stakeholders in AMR innovation towards the developments of new antibiotics, alternatives to antibiotics and diagnostics for human use.*
- *Stay up-to-date with ongoing research and development both in a public and private setting. Here, the RIVM and the Direction Medicines and Medical Technology can be partner, as they already play an important role in the signaling of various biotechnological developments.*
- *If decided upon to be necessary, match healthcare demand, market demand, and ongoing research. The network that they create (consisting out of both public and private players) facilitates easy access to this type of information.*

All while aiming at safeguarding both public goals and public interests.

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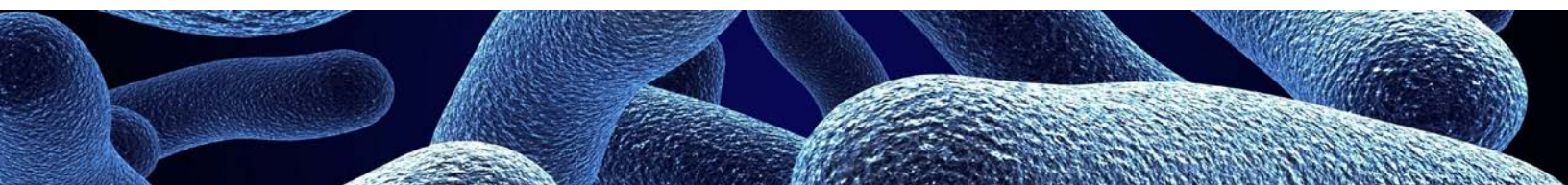


APPENDIX I – KNOWLEDGE DOCUMENT

APPENDIX I.1. ADDITION TO CHAPTER 4 – ANTIMICROBIAL RESISTANCE

THE PROBLEM WITH RESISTANCE

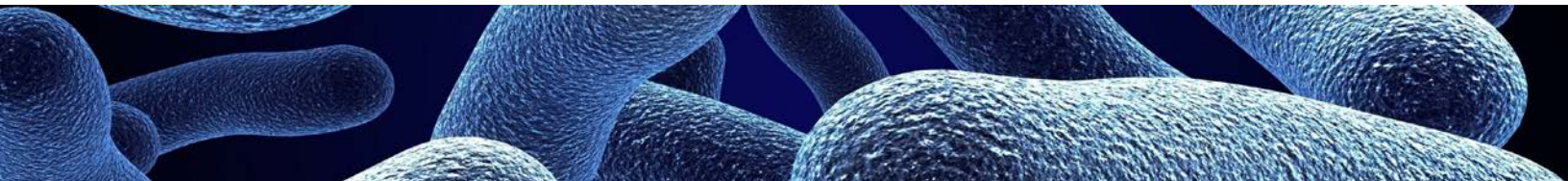
Antibiotic resistance is not new. Bacteria have been combatting each other using antibiotics since their existence, exemplified by the presence of several resistance genes in ancient permafrost [174]. This way, they have developed a large arsenal of antibiotic resistance genes, present in both pathogenic and non-pathogenic bacteria, called the resistome [77]. The average bacterium has resistance against 7-8 different antibiotics, with various modes of action [175]. Although the development of resistance is a natural phenomenon, selective pressure from antibiotic use in the treatment of human and animal infections and in agriculture has caused an increase in the evolution and spread of these resistance markers [176]. Indeed, a high correlation between antibiotic use in outpatient settings[2] and resistance is seen (Figure 29) [177]. Antibiotics inhibit the susceptible bacteria, while selecting for the resistant ones.

Initially, drug-resistant bacterial strains appeared in the places where most antibiotics were used, namely hospitals [178]. For example, soon after the introduction of penicillin in the 1940s, penicillin-resistant *S. aureus* emerged in hospitals in London [179]. Almost a decade later, multidrug-resistant pathogens were detected, including *E. coli* and *Shigella* [180]. Now, we have more than 15 different classes of antibiotics, all affecting processes and functions in the bacterial cell, and resistance mechanisms have been detected against all of them [181]. In general, resistance will occur if it is biochemically possible. However, it has to be noted that the vast majority of microbes do not cause disease, living peacefully with us and on us. Nevertheless, also these microbes are continuously exposed to toxic small molecules, including antibiotics, and have thus developed resistance towards them [66].

Types of resistance

There are three types of antibiotic resistance, namely intrinsic, adaptive and acquired resistance[176]. Intrinsic resistance is the ability of a bacterium to resist the activity of an antimicrobial agent through its inherent characteristics. An example of which is the presence of efflux pumps, actively transporting the antibiotics out of the cell. Adaptive resistance is the relatively less studied type of resistance. Here, the bacterium can temporarily become resistant towards an antibiotic, via alterations in gene and/or protein expression. It occurs in response to exposure to an environmental trigger, such as stress or nutrient conditions, and generally reverts when the inducing condition is removed.
Lastly, acquired resistance takes place when horizontal gene transfer (HGT) causes the passing on of resistance genes between bacteria, or when mutations in the DNA of the bacterium takes

[2] *Inpatient settings refer to the hospital; an inpatient procedure requires the patient to be hospitalized. Outpatient settings are for example the doctor's office or the emergency room.*



place, causing resistance [92, 176]. Indeed, drug resistance is mobile, the various resistance genes can be exchanged between bacteria from different taxonomic and ecological groups [181]. Although these genes usually confer resistance against a single family or type of antibiotic, more resistance genes can accumulate in a single organism [182].

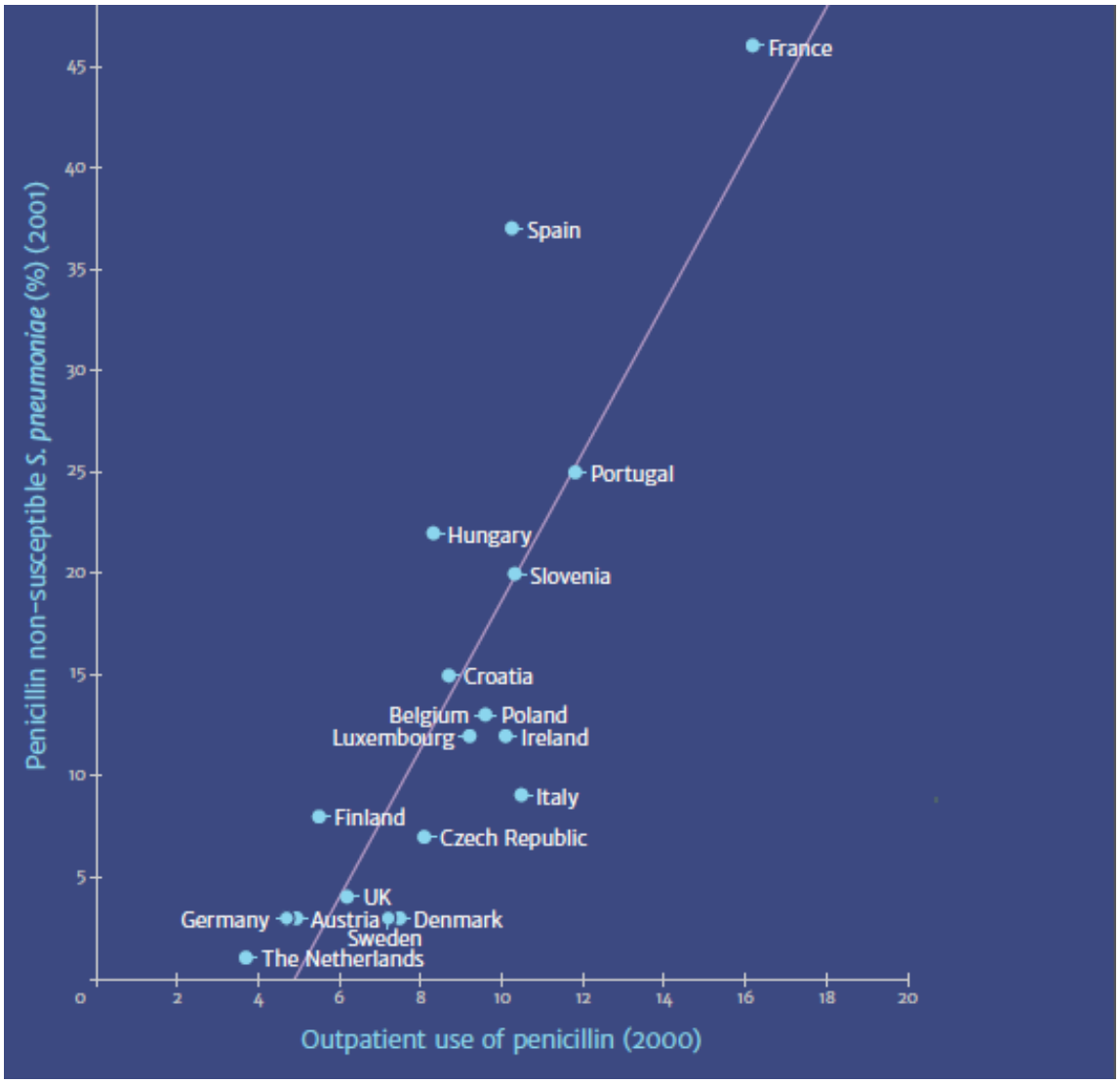
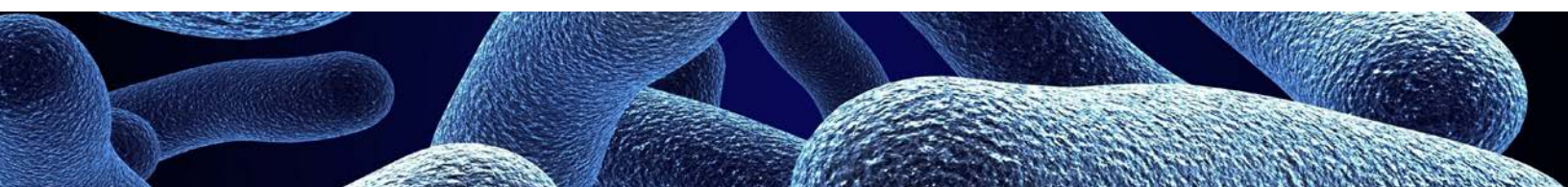


FIGURE 29: CORRELATION BETWEEN PENICILLIN RESISTANT S. PNEUMONIAE AND OUTPATIENT USE OF PENICILLIN IN EUROPE. ADAPTED FROM [98].

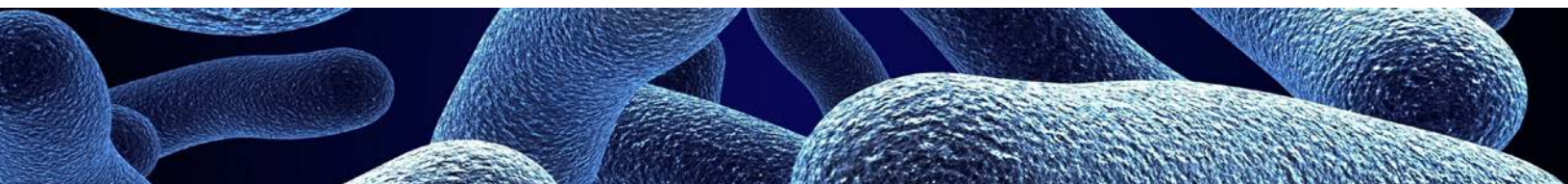


CLASSIFICATION OF ANTIBIOTICS

Antibiotics can be classified in various ways. One classification is based on whether they induce cell death (*bactericidal)* or whether they only inhibit cell growth (*bacteriostatic).* In addition, some antibiotics display bacteriostatic activity in some circumstances, while displaying bactericidal activity in others [183]. Another classification scheme is based on their bacterial spectrum (broad- versus narrow-spectrum antibiotics). The most useful method of is based on the cellular component or system they affect and thus how they function, for example the inhibition of bacterial cell wall synthesis, their DNA or RNA synthesis, or their protein synthesis. The major classes include the β-lactams, tetracyclines, aminoglycosides, macrolides and the synthetically produced (fluoro)quinolones. In addition, antimicrobial peptides, produced by bacteria, have been proposed as a replacement for antibiotics as well. (Table 9).

Antibiotic class	Example(s)	Mechanism of action	Type of activity
(Fluoro)quinolone		Inhibition of DNA replication	Bactericidal
Aminoglycoside	Streptomycin	Inhibition of protein synthesis	Bactericidal
Glycopeptide	Vancomycin, teicoplanin.	Inhibition of cell wall synthesis	Bactericidal
Lipopetide	Daptomycin	Inhibition of cell wall synthesis	Bactericidal
Macrolide	Erythromycin	Inhibition of protein synthesis	Bacteriostatic
Rifamycine	Rifampin	Inhibition of RNA synthesis	Bactericidal
Tetracyclin	Tetracycline, doxycycline	Inhibitions of protein synthesis	Bacteriostatic
β-lactam	Penicillin, cephalosporin, carbapenem	Inhibition of cell wall synthesis	Bactericidal
Bacteriocin	Lantibiotics	Inhibition of cell wall synthesis and membrane pore production	Bactericidal or bacteriostatic, depending on the bacteriocin.

TABLE 9: CLASSIFICATION OF ANTIBIOTICS.



HOW DO ANTIBIOTICS WORK?

Antibiotics kill or inhibit the growth of their target bacteria in different ways. Upon the interaction of the specific antibiotic with the target, an essential function of the bacterium is inhibited. These bacterial responses are generally well-characterized, and fall into four categories: inhibition of DNA, RNA, protein synthesis or cell wall synthesis [118]. In addition, a general cell response towards all classes of bactericidal antibiotics has also been shown, depending on a drug-induced stress response involving the production of harmful hydroxyl radicals [184].

Inhibition of DNA replication (quinolones)

The synthetically synthesized quinolones, including the fluoroquinolones, function by inhibiting the replication of DNA. The antibiotics' primary targets are the topoisomerases. These enzymes regulate the unwinding and overwinding of DNA by cutting the phosphate backbone of DNA and rejoining it, in order to allow its replication and transcription [185]. The quinolones target topoisomerase II and IV, inhibiting the progression to the rejoining step, trapping these enzymes at the DNA cleavage stage. The double-stranded breaks that generated this way, prevent the DNA replication machinery continue, resulting in inhibition of DNA synthesis and eventually cell death [186] (Figure 30).

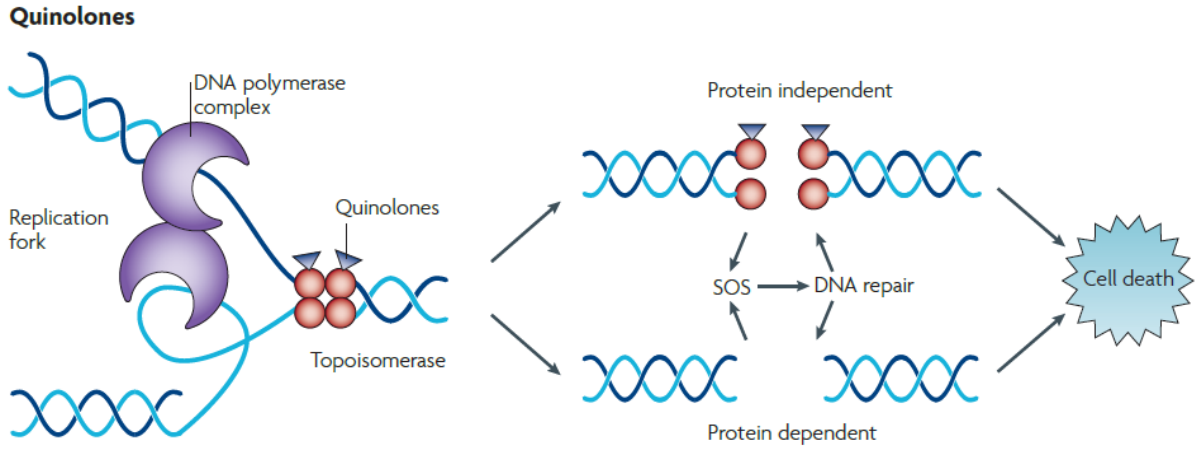
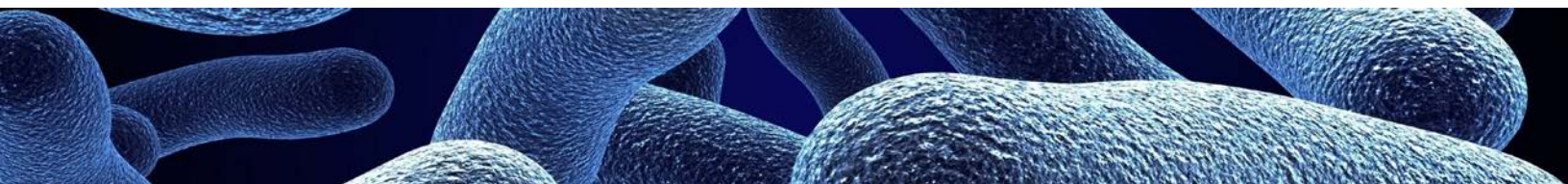


FIGURE 30: MECHANISM OF ACTION OF THE QUINOLONE CLASS OF ANTIBIOTICS. ADAPTED FROM [118].

Inhibition of RNA synthesis (rifamycines)

The rifamycine class of antibiotics functions by inhibiting the DNA-dependent RNA synthesis. More specifically, it inhibits RNA polymerase, by binding to the β-subunit of a DNA-bound and actively transcribing RNA polymerase [187, 188]. This β-subunit is located in the channel of the complex formed by the binding of RNA polymerase and the bacterial DNA, where the newly formed RNA strand emerges [189].



The inhibition of cell wall synthesis (β-lactams, glycopeptides, lipopeptides, lantibiotics)

The peptidoglycan layers that surround the bacterial cell contain glycan and peptide strands, which are enzymatically cross-linked. These peptide cross-links provides mechanical strength, which protects the bacteria from osmotic pressure forces that could kill the bacteria [183]. β-lactams and glycopeptides affect steps in the bacterial cell wall biosynthesis, causing changes to the cell shape and size, inducing stress responses and ultimately lead to cell death via a process called lysis. The lysis of cells occurs when the cell envelope ruptures, causing the intracellular components to flow into the surrounding environment of the cell [118].
β- lactams inhibit the function of penicillin-binding proteins (PBPs). These enzymes are, together with the transglycosylases, responsible for the maintenance of the peptidoglycal cell wall, via synthesis and remodeling [71]. In addition, these antibiotics induce a toxic malfunctioning in the cell-wall synthesis machinery [190]. Glycopeptide antibiotics reduce the mechanical strength of the cell, by binding to the peptidoglycan units, making them unavailable to the transpeptidases and PBPs [191]. Lipopeptide antibiotics, such as daptomycin, insert themselves into the cell membrane and induce membrane depolarization, affecting its structural integrity [118].

Inhibition of protein synthesis (macrolides, lincosamides, streptogramines, amphenicols, oxazolidinones, tetracyclines and aminoglycosides)

After RNA synthesis, mRNA is translated into proteins, involving the ribosome over three sequential phases: initiation, peptide elongation and termination processes. The bacterial ribosome is a two-subunit nucleoprotein particle, existing out of the 50S and the 30S subunit. Some antibiotics block processes at the 50S ribosome, while others act at the 30S ribosome (Figure 31) [183]. In general, the ribosome is an ideal target for antibiotics, as bacteria are unable to grow without them.

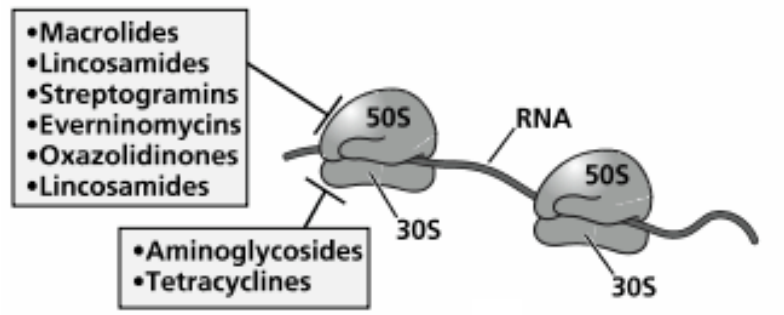
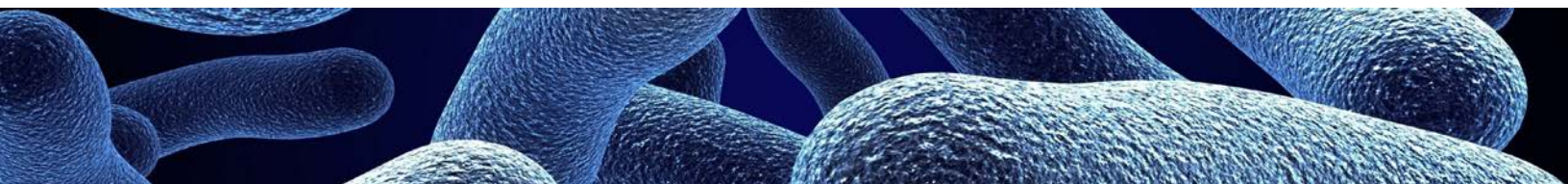


FIGURE 31: ANTIBIOTICS THAT BLOCK BACTERIAL PROTEIN SYNTHESIS. ADAPTED FROM [183].

50S antibiotics include the macrolides, lincosamides, streptogramines, amphenicols and oxazolidinones. They inhibit the 50S ribosome by physically blocking either the initiation of protein translation, or the translocation of peptidyl transfer RNAs (tRNAs). These tRNAs serve as the physical link between amino acids and mRNA, and are thus required for translation. Blocking the translocation of these tRNAs inhibits the elongation of the peptide chain [192] .



30S antibiotics include the tetracyclines and aminoglycosides. Tetracyclines block the binding of the incoming tRNAs to the ribosome [193]. Aminoglycosides on the other hand, bind to the 30S subunit, inducing misincorporation of amino acids into elongating peptides. Subsequently, these mistranslated proteins misfold and incorporate themselves into the cytoplasmic membrane, increasing cell permeability. This allows increased uptake of the drug, resulting in increased ribosome inhibition and ultimately, cell death (Figure 32) [118, 194].

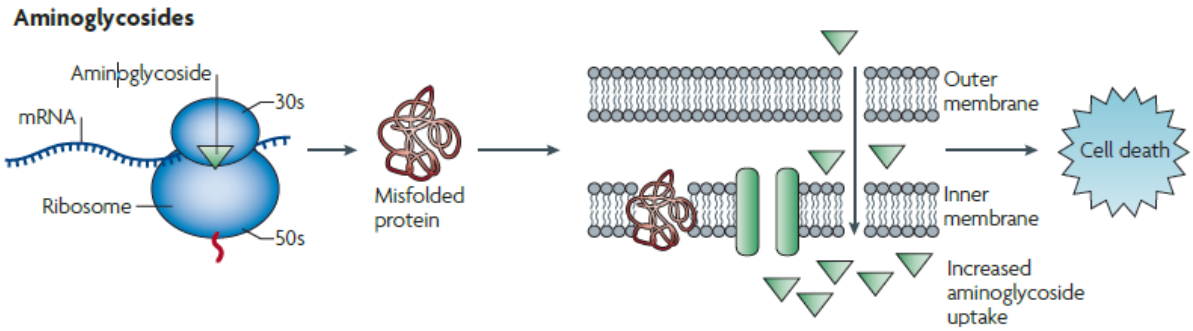
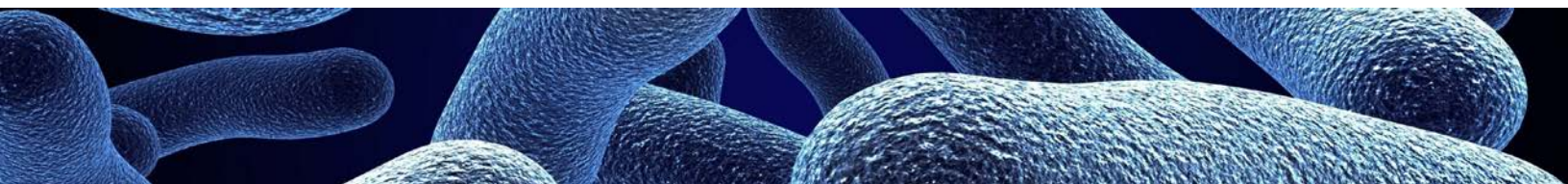


FIGURE 32: MECHANISM OF ACTION OF THE AMINOGLYCOSIDE CLASS OF ANTIBIOTICS. ADAPTED FROM [118].

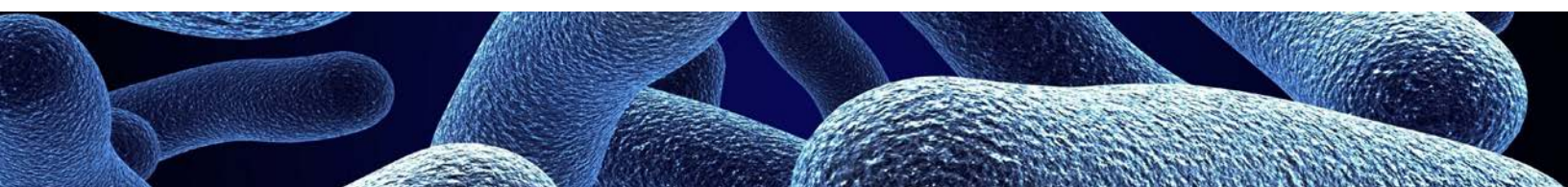


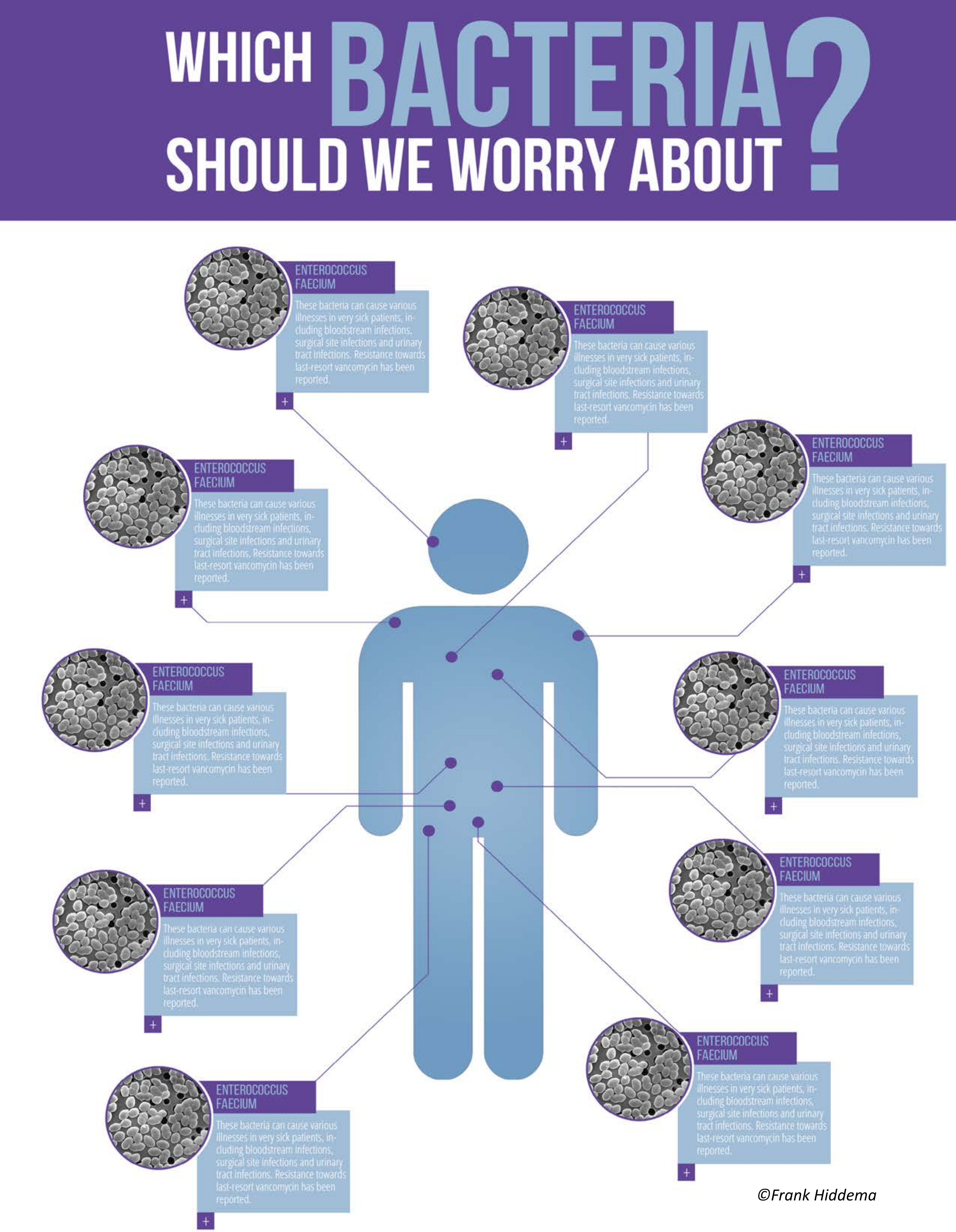
WHICH BACTERIA SHOULD WE WORRY ABOUT?

Every day of our lives, we live together with various microorganisms. They are an intrinsic part of us and outnumber human cells approximately by ten times [195]. Most of them reside in the gut and on the skin, and are harmless or even beneficial. However, several species are pathogenic, and especially in hospitals, where patients generally have a comprised defense against bacterial diseases, these are not as welcome. Major concerns are caused by the increasing emergence of multidrug resistance in the so-called ESKAPE pathogens: ***E****nterococcus faecium,* ***S****taphylococcus aureus,* ***K****lebsiella pneumoniae,* ***A****cinetobacter baumannii,* ***P****seuodomonas aeruginosa and* ***E****nterobacter* spp. [80]. These pathogens are not only important because they comprise the biggest part of the nosocomial infections, but also because they have many different forms of transmission, pathogenesis and resistance [196]. In addition, pathogens that cause food-borne diseases, such as *Escherichia coli, Salmonella* and *Clostridium difficile,* and the pathogens *Mycobacterium tuberculosis* and *Neisseria gonorrhea* can be added to this list [197].

The US Centers for Disease Control and Prevention (CDC) has highlighted three “urgent” resistance threats: carbapenem- resistant Enterobacteriaceae (including *E.coli* and *Klebsiella spp.*), *Neisseria gonorrhoeae* (causing gonorrhea) and *Clostridium difficile* (causing life-threatening diarrhea). In addition, a dozen “serious” threats were outlined (such as methicillin-resistance *S. aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE)) [198].

Recently, worrying evidence was found of resistance for the last-resort antimicrobial colistin, in both *Salmonella* and *E.coli.* In addition, resistance to the antimicrobial ciprofloxacin, important in the treatment of human infections, was found in *Campylobacter,* together with *Salmonella,* a key player in food poisoning [199].





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THE ESKAPE PATHOGENS

Enterococcus faecium

Enterococci are Gram-positive bacteria and can cause various illnesses among very sick patients in healthcare settings, including bloodstream infections, surgical site infections and urinary tract infections. Some *Entercoccus*, especially *E.faecium* have been reported to be resistant against the last-resort antibiotic vancomycin (VRE).

Staphylycoccus aureus

The Gram-positive *Staphylococci* are the most common cause of hospital-acquired, or nosocomial infections[200] and are predominant members of the human skin microbiota [201]. High rates of methicillin resistant *S. Aureus* (MRSA) are seen, which can cause a range of illnesses, including skin and wound infections, pneumonia and bloodstream infections. The resistance causes the treatment of common skin and wound infections to rely on second-line drugs in many countries [55].

Klebsiella pneumoniae

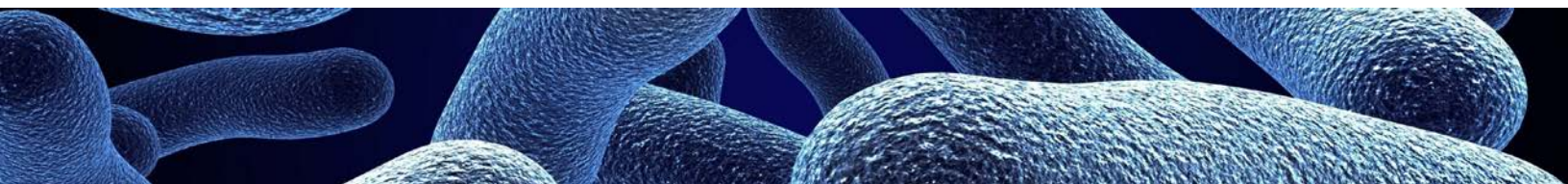
K. pneumoniae are Gram-negative bacteria which normally reside in the gut. Together with the *Enterobacter* species, *Escherichia coli, Klebsiella* and *Salmonella*, it is part of the family of Enterobacteriaceae. In healthy individuals, *K.pneumoniae* generally do not cause infections. However, in immune comprised patients, they can cause urinary and respiratory tract infections. They have been reported to have an increased resistance towards 3rd generation cephalosporins, causing the treatment of infections caused by these bacteria to rely on the last-resort antibiotics carbapenems. Concerning is the fact that resistance towards these antibiotics is also seen more and more, with proportions of resistance up to 54% reported (carbapenem-resistant Enterobacteriaeceae; CRE) [55]. In addition, extended-spectrum β-lactamase (ESBL) producing Enterobacteriaecease , including *K. pneumoniae* and *E.coli*, have been detected. These bacteria are resistant against strong antibiotics including cephalosporins.

Acinetobacter spp.

Acinetobacter is a type of Gram-negative bacteria that can cause pneumonia or bloodstream infections in critically ill patients. Some strains are resistant to nearly all or all antibiotics, including last-resort carbapenems. About 63% of *Acinetobacter* is considered multidrug-resistant and at least three different classes of antibiotics can no longer cure these infections [140].

Pseudomonas aeruginosa

These Gram-negative bacteria are often described as opportunistic pathogens, colonizing the gastro-intestinal and respiratory tract, causing disease in patients who already have a compromised immune system and/or an underlying disease [183, 202]. *P.aeruginosa* are among the leading causes of nosocomial infections, such as pneumonia, bloodstream infections, urinary tract infections and surgical site infections. Some strains have been found to be resistant to nearly all or all antibiotics.



Enterobacter spp.

Enterobacter species are Gram-negative bacteria and most commonly cause urinary and respiratory tract infections, but can also cause bloodstream infections. They can produce ESBL and carbapenemases, causing them to be resistant against various antibiotics [203] .

OTHER IMPORTANT BACTERIAL PATHOGENS

Escherichia coli

A high incidence of resistance is seen in the Gram-negative E.coli bacteria, more specifically towards the 3rd generation cephalosporins. These bacteria are the leading cause of urinary tract infections, and some types can cause severe intestinal infections among others [204]. Infections most likely caused by these bacteria, often rely on treatment with carbapenems, the last resort antibiotic in the treatment of severe community and hospital- acquired infections [55].

Salmonella

Salmonella are Gram-negative bacteria that can cause the food-borne disease Salmonellose. Symptoms include diarrhea, fever and abdominal cramps. In addition, some infections can spread to the blood and can have life-threatening complications. Resistance against the most common antibiotics used for complicated infections, including ceftriaxone (a cephalosporin) has been reported [140].

Neisseria gonorrhoeae

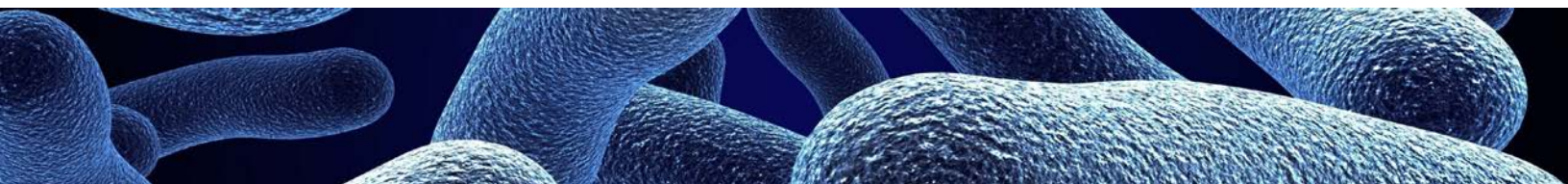
These Gram-negative bacteria are the cause of gonnorhea, a sexually transmitted disease that can cause inflammation at the urethra, cervix, pharynx, or rectum. Resistance towards tetracycline is seen, as well as a reduced susceptibility towards the first-line treatment drug ceftriaxone [140]. In the Netherlands however, no resistance towards ceftriaxone has been reported in 2014 [205].

Clostridium difficile

Clostridium difficile is a Gram-positive bacterium, causing life-threatening diarrhea. Infections occur mostly in patients that have had a recent medical treatment and antibiotics. It can spread rapidly and a strain resistant to fluoroquinolone antibiotics, commonly used to treat other infections, has been detected [140]

Mycobacterium tuberculosis

Tuberculosis, the disease caused by *Mycobacterium tuberculosis,* is one of the most common infectious diseases and frequent cause of death worldwide. Due to its waxy coating on the cell surface, the bacterium can appear both as Gram-positive and Gram-negative. The bacteria can spread through the air and can affect any part of the body, although most often the lungs. Resistance towards first-line drugs has been detected, which causes the treatment to be more complex, timely and expensive.



APPENDIX I.2. ADDITION TO CHAPTER 5.1. – NOVEL ANTIBIOTICS

DEVELOPING NOVEL SMALL-MOLECULE ANTIBIOTICS

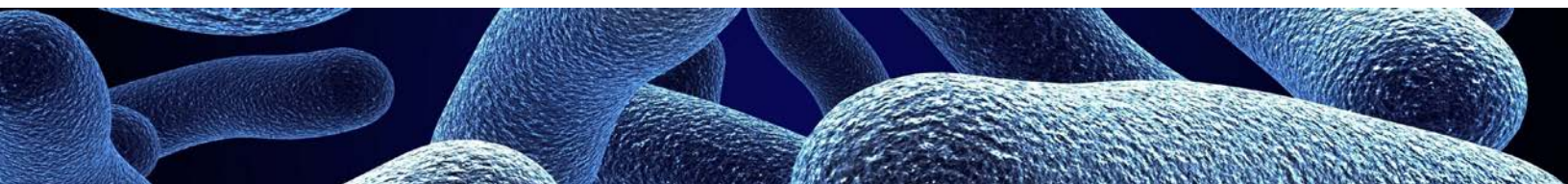
The class of small-molecule antibiotics includes both natural products and synthetic molecules, and is the largest class of antibiotics. Environmental microbes create the natural products using secondary metabolic pathways to assemble simple building blocks into complicated structures [92]. These pathways possess modularity, an important feature in the field of synthetic biology[206].

Polyketides

One example of using the synthetic biology approach to develop novel small-molecule antibiotics, is using the biosynthesis pathway of polyketides. The polyketide class of secondary metabolites, which include macrolide and tetracycline antibiotics, are produced by polyketide synthases (PKS). This family of large enzyme complexes can be classified into three groups. However, they all follow a similar path in the assembly of these molecules. These pathways are present in Actinomycetes , which are large producers of natural antibiotics[92]. The genes responsible for the biosynthesis of polyketides are clustered in BGCs. Each BGC encodes the PKS responsible for the creation of the backbone, together with the tailoring enzymes. In addition, it encodes the regulation of the BCG and resistance to the end product [207]. Microbial genome sequences can be screened in order to detect for these uncharacterized secondary metabolite BCGs. Sequence analysis software, such as antiSMASH, provides synthetic biologists to do this in a highly automated fashion [90]. It also enables the discovery of cryptic or silent BCGs, of which the corresponding products are not produced in detectable levels in normal culture conditions. This process is regulated by complex control circuitries, which is difficult to circumvent. By adding new synthetic regulation via the refactoring process, libraries of BCGs can be generated, which leads to the biosynthesis of novel PKS [207].

DEVELOPING NOVEL PEPTIDE ANTIBIOTICS

Another major class of antimicrobials is represented by peptides. Peptide antibiotics fall into two classes: the non-ribosomally synthesized peptides (NRPs) and antimicrobial peptides (AMPs). Whereas NRPs are also produced by environmental microbes, AMPs are produced by multi-cellular organisms as part of their immune system [92]. Using glycopeptides as an example, the synthetic biology approach to producing novel NRPs will be explained. For AMPs, the production of indolicidin will illustrate this process. Peptide antimicrobials provide researchers with more opportunities to create novel molecules than polyketides, as both natural and unnatural amino acids (the building blocks of peptides) can be used.



NRPs: Glycopeptides

A large percentage of the natural peptide antibiotics are produced by non-ribosomal peptide synthases (NRPSs). Non-ribosomal peptides (NRP) have a comparable logic to polyketides when it comes to the modular organization of their assembly line [208].

Glycopeptide antibiotics (GPAs), such as vancomycin and teicoplanin, are important antibiotics that target Gram-positive bacteria. The BCGs of these antibiotics encode for the NRPs that are required for the assembly of the peptide scaffold, and they carry the genes that encode for self-resistance, export and tailoring enzymes [209]. GPA tailoring modifications can either be primary, or secondary. Primary modifications directly modify the amino acids of the scaffold, whereas secondary modifications tailor the primary modifications [75]. A synthetic biology approach in a heterologous host as described above can produce large libraries of GPAs that can be screened for antibiotic activity.

AMPs: Indolicidins

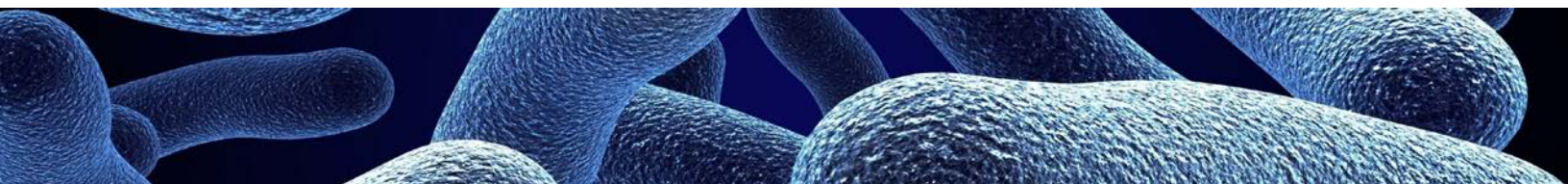
As mentioned, AMPs are defense peptides that are part of the innate immune system of plants or animals. They have a more general mechanism in targeting bacteria, disrupting their membrane [210]. Engineering novel AMPs is different from engineering natural secondary metabolite pathways. Because of their small size, and general properties in disrupting the cell wall, they can be easily used in computer-simulation. Tsai et al. used this approach to study the molecular mechanisms of the AMP indolicidin, after which they could design a synthetic variant with improved properties [211]. The bacteria however, could degrade AMPs, although this can be circumvented by changing their chemical properties. In addition, the bacteria could acquire resistance towards these molecules [92]

NON-TRADITIONAL ANTIBIOTICS: BACTERIOCINS

Together with the production of antibiotics, bacteria also produce antibiotic peptides called bacteriocins. Bacteriocins naturally occur, and are normally produced by bacteria under stressful conditions. They are toxic to their closely related neighboring bacterial strains, but not to their host strain [102, 212]. Bacteriocins can generally be divided into different groups, of which the group of lantibiotics will be used as an example below.

Lantibiotics

The lantibiotics (*lan*thionine-containing *antibiotics*), of which the most well-known example is nisin, were discovered in the 1920s. Nisin, produced by *Lactococcus lactis,* inhibits bacterial peptidoglycan synthesis and creates membrane pores [78]. It has been used by the food industry as a preservative for decades, but no significant resistance has yet been observed. However, resistance has been observed *in vitro* [213]. Many lantibiotics show activity against a variety of Gram-positive bacteria, and some against multidrug-resistant bacteria, such as MRSA and VRE [214].



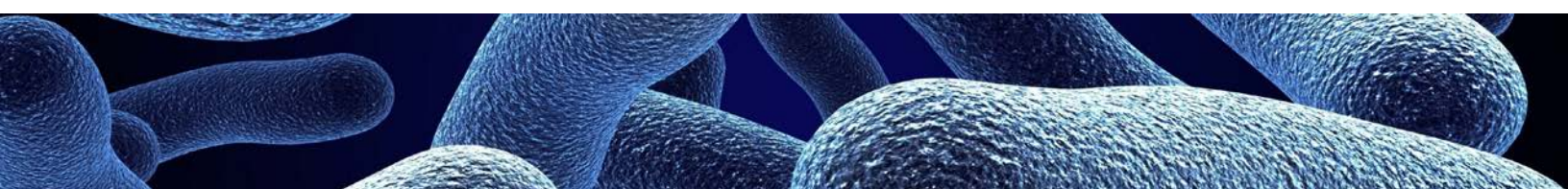
Despite these important abilities, they have not been used extensively as therapeutics. This is mainly due to their expensive and time-consuming manufacturing, and their injection, rather than their oral administration [215]. Until now, one type of lantibiotics has entered phase 1 clinical trials for use against *Clostridium difficile*, showing good results so far [216]. Lantibiotics are small post-translationally modified antimicrobial peptides produced by Gram-positive bacteria [217]. Lantibiotics disrupt cell wall synthesis and can create membrane pores, by binding to lipid II, an essential and highly conserved molecule. All lantibiotics have a leader peptide or sequence. This leader sequence can be required for secretion, or it can prevent activation of the lantibiotic until it is outside of the cell. In the latter case, the leader peptide is eventually cleaved off, thereby releasing the active lantibiotic [218].

The synthetic biology approach

Synthetic biology offers tools to create new lantibiotics, with improved properties as compared to natural lantibiotics. In addition, it can offer solutions to the upscaling of the production of lantibiotics, which is generally a problem. Synthetic biology could play a role in the optimization of production pathways [Appendix II. 4]. The traditional approach of finding bacteriocins is based on the isolation of a potential producer organism and the screening for antimicrobial activity in the extracellular milieu, where these peptides are secreted. Using high-throughput screening , novel bacteriocins from the cultured bacteria were then discovered. In addition, via organic synthesis or semi-synthetic synthesis, their properties can be further improved [213]. However, there are many lantibiotic gene clusters that are not expressed in common growth media, the so-called 'silent lantibiotics'. It has been shown that lantibiotic biosynthesis enzymes can modify virtually any substrate peptide, as long as it is fused to the nisin leader peptide [219]. Modularity, one of the characteristics of synthetic biology, is also seen in the peptide structure of lantibiotics. Once a combinatorial DNA library of these modules was made, synthetic DNA was used to produce these different modules. To achieve this, a context-insensitive post-translational machinery is implemented into a production chassis. This way, a variety of novel lantibiotics can be produced by the bacteria. Subsequently using high-throughput screening methods, these products can be screened for antimicrobial activity [213]. A disadvantage of lantibiotics, and bacteriocins in general, is the fact that they are not developed in humans, but in the niche of bacteria. Therefore, they have developed resistance mechanisms against these molecules. The perceived benefit of new-to-nature molecules developed with synbio is the fact that no specific resistance mechanism has been developed against them. However, bacteria will probably acquire these mechanisms, although the aim of synbio is that resistance develops slower than usual [Appendix II.4.].

Expanding the genetic code of lantibiotics

To expand the repertoire of lantibiotics even further, non-canonical amino acids can be used for lantibiotic synthesis. Normally, only 20 amino acids, the canonical amino acids, are used by organisms to build peptides. By re-allocating one or more specific codons, the genetic code can be expanded. This way, lantibiotics can be produced with chemical properties not developed by natural evolution [220]. Even though this is possible (in two ways, explained in Appendix II.4), not many research has so far been done in this area.



APPENDIX I.3. ADDITION TO CHAPTER 5.2.1. – PROBIOTICS: SENSE AND DESTROY

TARGETING VIBRIO CHOLERAE

Another approach using engineered *E.coli*, focused on infection by the bacterium *Vibrio cholerae* [221]. *V. cholerae* resides in the gastro-intestinal tract, and produces virulence factors, such as the cholera toxin, which causes the symptoms of cholera. Normally, it produces the signaling molecule CAI-1 once it senses its own critical density, after which the production of the virulence factors is inhibited. Feeding mice with probiotic *E.coli* engineered to express CAI-1, before the ingestion of *V.cholerae,* reduced the amount of the pathogenic bacteria and thus the production of cholera toxin (Figure 33). This resulted in an increased survival rate 48 hours after infection, from 0% to 92%. Even though this is a prophylactic method, it might be an inexpensive application to prevent cholera infection in the developing world. One of the challenges that remains in using these types of engineered probiotics in clinical settings, is the stable long-term colonization of these bacteria. In addition, engineering a robust function of synthetic gene networks in an *in vivo* setting is challenging as well [124].

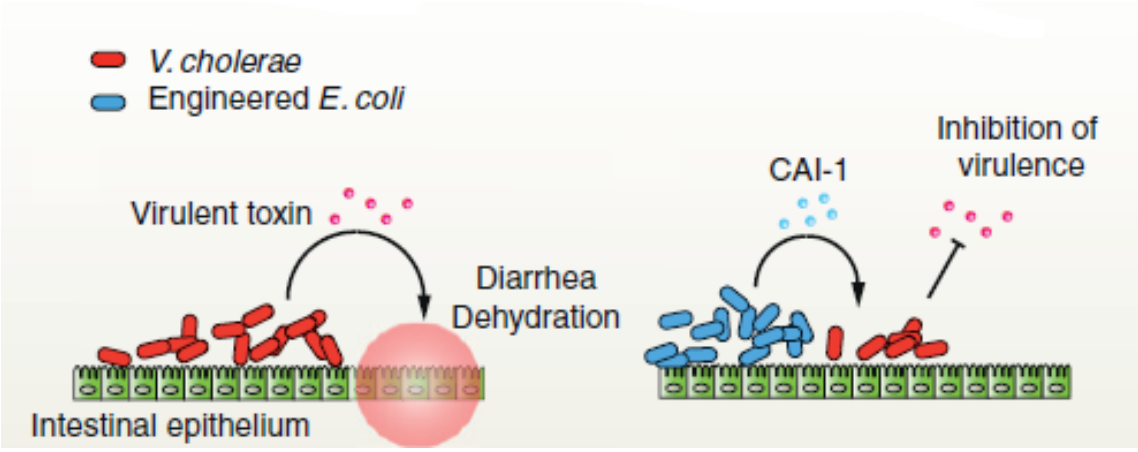
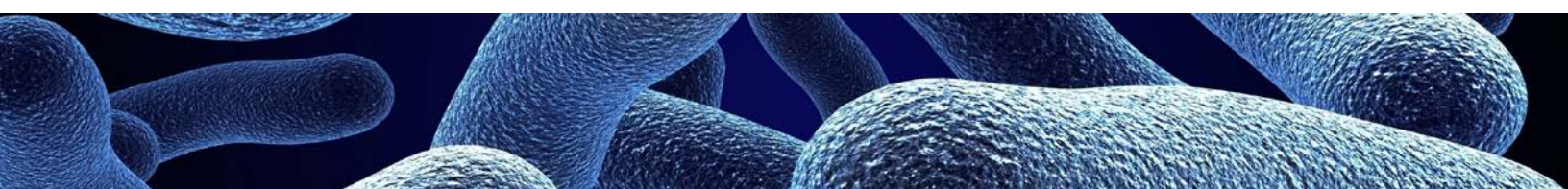


FIGURE 33: ENGINEERED *E.COLI* REDUCED THE AMOUNT OF COLONIZED *V.CHOLERAE* AND INHIBITED VIRULENCE. ADAPTED FROM [222].

AN EXAMPLE FROM THE IGEM COMPETITION: THE LACTOAID

In 2014, iGEM Groningen developed a new kind of bandage, in order to prevent wound infections by *S.aureus* or *P. aeruginosa* [223]. This way, the use of antibiotics after an infection could be reduced. To this end, an engineered strain of *L. lactis* was placed in a hydrogel, together with various nutrients. *L. lactis* was able to detect the quorum sensing molecules of both pathogens, and subsequently produce the antimicrobial peptide nisin, together with other Infection-Preventing-Molecules (IPMs). After placing the bandage on the wound, it can be activated by breaking the water pockets, initiating the growth of the engineered bacteria.



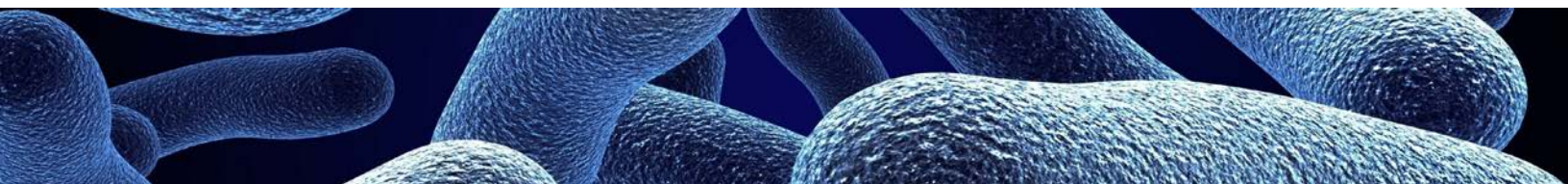
APPENDIX I.4. ADDITION TO CHAPTER 5.2. ENGINEERED BACTERIOPHAGES

ADVANTAGES AND DISADVANTAGES OF PHAGE THERAPY

As mentioned, the specificity of phages towards bacteria provides benefits for the normal flora, letting it remain intact and protecting the bacterial species that are not part of the phages host range. On the other hand, treatment with phages also causes certain disadvantages as compared to antibiotics. For example, the pathogen that causes the infection always has to be identified, to know which bacterial species to target [224].

So far, lytic phage therapy has shown success mostly in external tissues and mucosal tissues, such as the skin, the gastrointestinal tract, the urogenital tract, upper respiratory tract, eyes or ears [115]. For example, clinical phase I and II studies have been completed successfully for the treatment of chronic *P. aeruginosa* ear infections in humans [225]. Their application in internal tissues however, faces a number of obstacles. Most of the phages entering the blood stream, are sequestered by the spleen or liver. In some phages, this barrier has been overcome by selecting for mutants that do not face this problem [226]. In addition, frequent use of phages creates an immunogenic response, leading to an antibody-neutralizing effect. Lastly, diffusion of the phages into the internal tissues from the bloodstream is problematic, due to their large size compared to antibiotics [115]. Because phages face these difficulties in their internal use, they are generally not seen as a complete alternative to our current antibiotics [Appendix II.3]

As with antibiotics, bacteria can develop resistance towards phages, although this propensity is generally lower [227]. Bacteriophages are an abundant species and co-exist with bacteria, therefore having an important impact on them [228]. Consequently, bacteria have developed a variety of resistance mechanisms towards these phages.
For example, bacterial restriction-modification (R-M) systems function are part of the bacterial innate immune system. The system can be horizontally acquired by bacteria and attacks foreign DNA entering the cell. The CRISPR-Cas system on the other hand, is part of the adaptive immune system of bacteria *(see text box 'The CRISPR/Cas system')*. Both systems cause the degradation of the injected phage DNA[229]. The use of cocktails of bacteriophages could reduce the risk of the development of resistance, as it is harder for bacteria to adapt themselves to different phages with different working mechanisms. In addition, because bacteriophages are dynamic, they are able to adapt to the bacteria as well. One way of doing so, relies on the selection of phages that do not have the part of the DNA for which the bacteria created a memory [Appendix II.3].



THE CRISPR/CAS SYSTEM

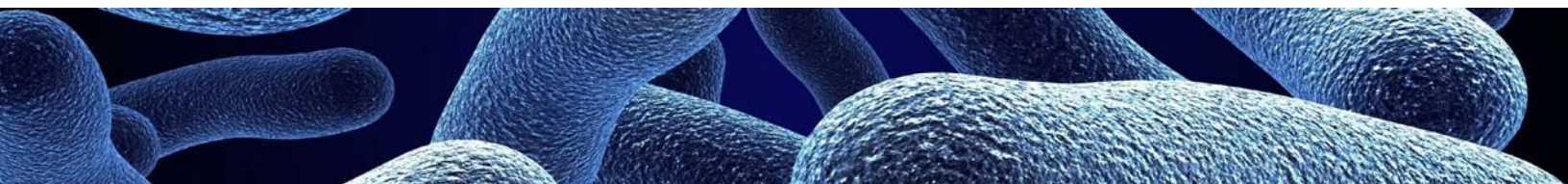
Clustered regularly interspaced short palindromic repeats (CRISPR)- Cas (CRISPR-associated) systems are used by many bacteria in order to cleave or degrade the genetic materials of, for example, invading phages or plasmids. After exposure to foreign DNA, a so-called 'spacer' is formed. These spacers specifically recognize the complementary DNA of foreign invaders, after which an endonuclease protein (Cas9) is guided to recognize and cleave the invading genetic material. These CRISPR-arrays have revolutionized molecular biology (and synthetic biology), as they are used to precisely engineer genomes and manipulate gene expression in various organisms [14].

SENSITIZATION TO ANTIBIOTICS: DELIVERY OF DOMINANT ANTIBIOTIC-SENSITIZING GENES

Another method using engineered phages relies on the delivery of dominant genes causing sensitivity to existing antibiotics. Temperate phages were used to transfer specific genes into bacteria and subsequently integrate them into the bacterial genome. The integrated genes provided sensitivity to two types of antibiotics, namely streptomycin and quinolones. This sensitization was achieved simply via the dominance of the sensitive allele over the resistant one. These sensitized bacteria were selected by linking the sensitivity genes to a gene conferring resistance to the toxic compound tellurite. Adding tellurite killed the bacteria with the sensitive genes, while leaving the bacteria with the dominant genes intact. Following this proof-of-principle stage, it was proposed that this method can be used to sensitize pathogens on nosocomial surfaces, as well as the natural flora residing on the skin and hands of hospital personnel. In addition, it could be sprayed in hospitals, thus gradually reversing the occurrence of drug-resistant pathogens on hospital surfaces and replacing resistant populations with a sensitive one. However, resistance towards these phages would likely to occur rapidly, as bacteria will probably find a way to remove the antibiotic-sensitive genes [Appendix II.3].

SENSITIZATION TO ANTIBIOTICS: DELIVERY OF A CRISPR-CAS SYSTEM

In another study, temperate phages were used to sensitize bacteria and selectively kill antibiotic-resistant bacteria [14]. The phages deliver a CRISPR-Cas system into the genome of antibiotic resistant bacteria. This CRISPR-Cas system destroys both the plasmids encoding antibiotic resistance and the genetically modified lytic phages. This means that the antibiotic-sensitized bacteria are also resistant towards lytic phages. In other words, they have a selective advantage, as the nonsensitized bacteria are killed by lytic phages. The CRISPR-array used targets conserved sequences of two resistance genes. These genes encode for β-lactamases that confer resistance to carbapenems, which are β-lactam antibiotics that are often used as a last line of defense against resistant pathogens [230].
This strategy can be applied for treating hospital surfaces and in hand sanitizers for targeting the skin flora of hospital personnel. Resistance towards the lytic phages by the non-sensitized bacteria can occur. This evolvement can be minimized however, by using a mixture of lytic phages. In addition, the probability of CRISPR-escaping mutants could be reduced by using multiple spacers.



APPENDIX II - INTERVIEWS

APPENDIX II.1. LIST OF INTERVIEW PARTICIPANTS

Policy

- Policymaker VWS (not included in this appendix).
- Gerard Dekker – Member of the management team long-term care VWS.

Funding

- Thera Habben Jansen – Program coordinator research program 'Antibiotic Resistance (ABR)'.

Academia

- Prof. dr. Oscar Kuipers –Head Molecular Genetics, University of Groningen.
- Dr. ir. Stan Brouns – Assistant professor Molecular Microbiology, University of Wageningen.
- Dr. Marnix Medema – Assistant professor Bioinformatics, University of Wageningen.

Investment company

- Junior investment professional at an investment company.

Network organization and service office

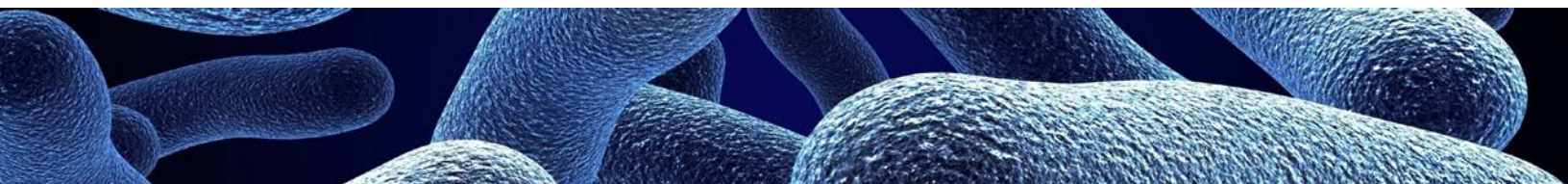
- Liana Steeghs – Managing director Immunovalley

Medical professionals

- Prof. dr. Marc Bonten – Clinical microbiologist, Head of the Department of Medical Microbiology at the UMC Utrecht, research leader of the theme 'Antimicrobial Resistance' of the NCOH.
- Prof. dr. Alex Friedrich – Clinical microbiologist, Head of the Department of Medical Microbiology at the UMC Groningen.

Other

- Dr. Laurens Landeweerd – Assistant professor at the Radboud University Nijmegen's Institute for Science Innovation and Society (Interview for critical reflection).



APPENDIX II.2. INTERVIEW THERA HABBEN JANSEN

Head of the research program ABR at ZonMw 16-02-2016

1. *How is the financing process for projects organized? In what way is determined which projects do and which ones do not receive funding?*
   This process goes through a few different steps. It started with informal contact with the client, the Ministry of Public Health, Welfare and Sports (VWS). ZonMw was asked to do a programming study, to map out a few different affairs:
   - The position of knowledge.
   - The current developments (e.g. if the political involvement is vast).
   - The possibilities for the Netherlands compared to e.g. Europe.

   From this, the 'knowledge agenda AMR' developed. Here, interviews were conducted with several stakeholders, amongst whom are scientists, the veterinary sector, funds, etc. These were then tested by the program committee. Research on policy documents from the WHO or Joint Programming Initiative on AMR (JPIAMR) is conducted, as well as surveys amongst project leaders of the current program.

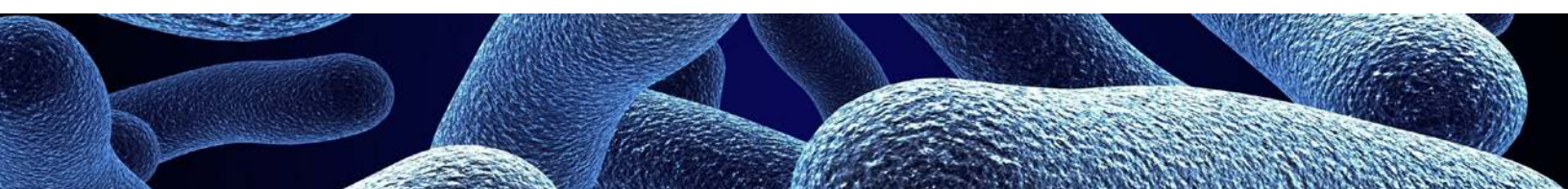
   From here, six research themes were extracted, which were then tested in an expert meeting with several stakeholders (amongst whom were a medical microbiologist, a big data expert, a behavioral scientist, an environment advice agency and representatives from the veterinary sector).

   In a meeting with approximately 60 stakeholders, possible research questions connected to these themes were determined.

   After drafting the knowledge agenda, AMR followed the formal assignment from VWS to set up a research program, ABR. This was based on:

   - The VWS policy letter (24-06-2015).
   - The strategic research agenda (SRA) by the JPIAMR.
   - The knowledge agenda AMR by ZonMw (2015).

   Here, emphasis was placed on the desire to have the research's outcomes contribute to the making of policy choices in preventing ABR in the next five years. Through this, research groups are already challenged to think about the applicability of their research. This does, however, leave little to no room for fundamental research within this program. If a new drug target were to be discovered, this research would then have to go through several phases before it would lead to a new antibiotic (this takes approximately 15 years). In addition, the chain for these new development is too small in the Netherlands, thus, European programs are needed here.



Four research themes followed from this:

- Mechanisms for the development and spread of ABR.
- Appropriate diagnostics.
- Mechanisms and targets for new antibiotics and alternatives to antibiotics.
- Optimizing antimicrobial therapy: dosage and use.

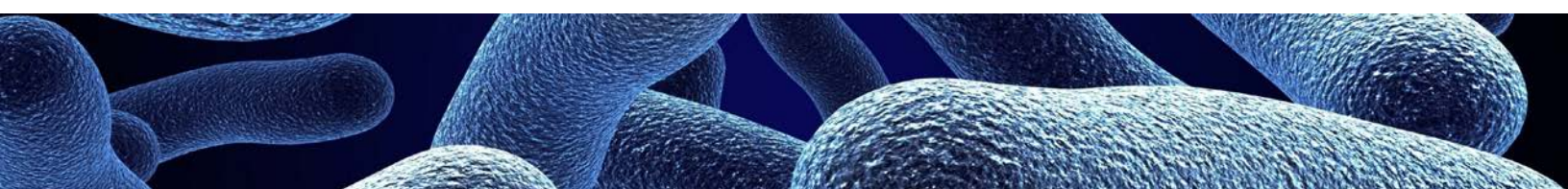
A formal assignment from VWS to ZonMw for the execution of the program 'Antibiotic Resistance (ABR)' followed. It was also noted that attention needed to be given to:

- Innovation
- Public-private partnerships
- Developments that lift barriers

These criteria, in addition to the general ZonMw criteria, have been taken into account when creating the text for the call. One of the program specific criteria concerns the granting of access to the data and the active sharing of the data (considering the fact that the data created through public funding).

2. *What could the possible barriers be?*
The use of diagnostics by general practitioners for example. An instrument to test for respiratory infections costs 20 to 25 euros per use, while treatment with antibiotics only costs 4 euros. For health insurance companies this is not an attractive business model. This could be a barrier for the use of diagnostics. Additionally, a general practitioner might want to avoid all risks with a certain patient and thus use an antibiotic that, considering the public's wellbeing and the development of resistance of bacteria, would have been better off not being used just yet.
The development of new antibiotics and its subsequent possible implementation into health care is another barrier. These are often left on the shelves, possibly because doctors are unaware of their existence, or because they want to keep it for a later time.

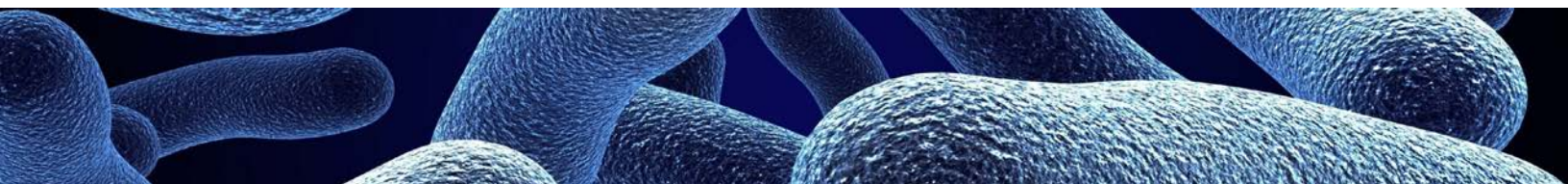
3. *What is the role of the program committee?*
The program committee gives scientific advice to ZonMw about project ideas and project applications. When the first round of subsidies opens up, the research groups have to deliver their brief application. The committee then gives a relevancy advice (on a five-point scale). The committee normally consists of 6 to 8 members, but due to the width of the field of research (behavioral scientists play a part, for example) and the relatively small amount of Dutch researchers in the AMR field (which could result in great involvement), there are 18 members in this program.



Three of the members, whose expertise connects well to the research proposal, are asked to give this relevancy advice. On this basis a research group can, even with a negative advice, put in a subsidy request. The committee can also give along advice, like cooperating with another group or a weak spot in the research. Around 100 to 150 advices are expected, with about 30 receiving a positive advice.

Next, an extensive subsidy request is put in by the research group, including for example the method and the budget. This is sent to three reviewers, mostly abroad. These reviewers are drawn form a large pool of about 500 to 600 scientists, so the selection process here is crucial. Additionally, inquiries are made for possible conflicts of interest, for example when the applicant has cooperated with the reviewer. Afterwards, a ranking of the research projects is made, and subsidies are assigned.

In short:
1. Creating a knowledge agenda:
    - Desk study.
    - Survey project leader.
    - Interviews.
    - Reviewing in expert meeting.
    - Reviewing in stakeholders meeting.
    2. Assignment for preparing a program:
    - Based on policy letter, SRA JPIAMR, knowledge agenda AMR.
    - In cooperation with the program committee AMR of ZonMw and VWS observers.
3. Approval of the program and assignment up to the execution:
    - Creating and opening up the first subsidy round.



APPENDIX II.3. INTERVIEW STAN BROUNS

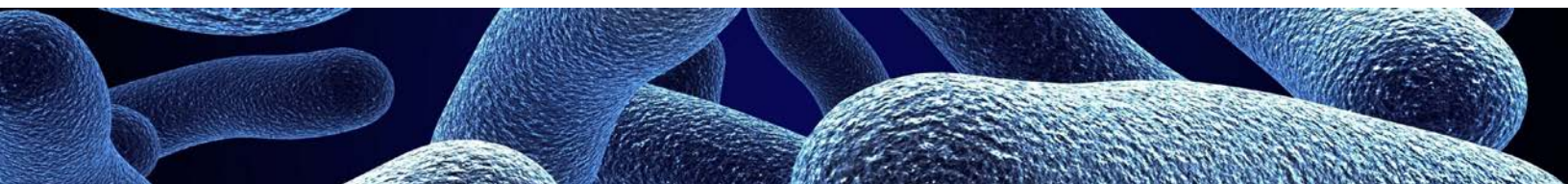
Assistant professor Molecular Microbiology at Wageningen University. 02-03-2016

1. *Could bacteriophages (engineered using synthetic biology, or not) scientifically speaking, be an alternative to our current antibiotics?*
   That is definitely a possibility. Scientifically, I don't see any problem with that. In Poland, Russia and Georgia, bacteriophages have been used successfully for 70 years in order to combat infectious diseases. However, I don't think bacteriophages can be used as a complete alternative to our current antibiotics. They cannot be used intravenously, and their use is thus limited to ectopic use, on for example biofilms on skin wounds.

2. *Which developments within the modification of bacteriophages (using synthetic biology) have the most potential according to you?*
   I think the use of endolysins has a good future perspective. They are trending and it is scientifically achievable. They aim at the peptidoglycan of a certain bacterium, and thus have a certain amount of specificity. A Swiss group, together with Micreos, is currently engineering endolysins as well.
   In addition, with every development, you have to consider if resistance could easily occur. If you for example introduce dominant antibiotic sensitive genes, the chances are big that the bacteria will export these genes out of the cell. The same goes for the use of srRNAs. It is also important to reach all bacteria, something that is difficult because bacteria can create a biofilm, or spores. Moreover, the space that is created in the population by removing a part of the bacteria, needs to be filled with other non-resistant bacteria. If some resistant bacteria remain, these could fill this void.

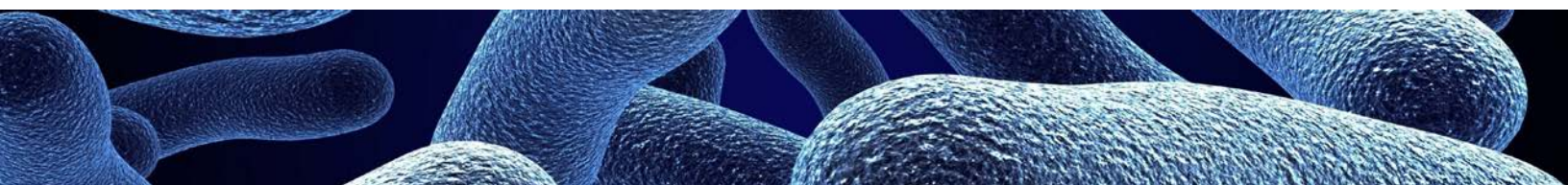
3. *What are the biggest barriers in the use of bacteriophages as an alternative to antibiotics?*
   In the Netherlands, this is probably mostly the laws and regulations. It has to go through several clinical trials under the Dutch Medicines Act, a process that could take years. There should be a different set of rules for bacteriophages. Scientifically speaking, bacteriophages could be used very quickly. In China for example, the legislation is more flexible, and they have progressed further with the developments regarding their use. For endolysins, it is easier to comply with the current legislation, because they are not 'alive'. The only issue is the fact that they cannot be extracted from a pathogen, but many other phages remain to retrieve the lysins from.

4. *Could bacteriophages that have been engineered using synthetic biology be used for qualitative and quick diagnostics?*
   Yes, in time, this would be possible. I think research is being done within field. You could equip phages with some sort of biosensor that could recognize certain sequences in bacteria. However, I think that currently, there are better and quicker methods, such as the direct sequencing of the DNA of the bacteria. In addition, the phage is dynamic, which can cause the range of bacteria that it recognizes, to change.



5. *Could bacteria become resistant towards phages?*
   Yes, that is possible, for example with CRISPR, which enables them to create a memory for the phages. On the other hand, bacteriophages are dynamic, causing them to be able to adapt as well. For example, they can select themselves for the phages that carry a protein that is able to shut down the CRISPR system, or the phages that do not have the peace of DNA that is stored in the memory of the bacterium. The use of phage cocktails can also aid in the prevention of resistance development. A bacterium can adapt less easily to phages with different working mechanisms.

6. *Are there any other risks associated to the use of phages adapted with synthetic biology? Can bacteria for example take in the genes that code for the toxins that were supposed to kill them?*
   In nature, DNA is constantly being exchanged between bacteria mutually, and between bacteria and phages, something we have known for a very long time. The exchanged DNA will remain there permanently if there's a selective benefit for the bacteria or phage associated with the DNA. If the toxin is very specific for the bacteria, there is no apparent reason to take over these genes. Many pathogenic bacteria have various endotoxins that are dangerous to humans. In choosing a strategy to kill bacteria, it is important to take into account the release of these endotoxins. It could therefore be smart to kill these bacteria from the inside out, in order to let the outside wall of bacteria remain intact. This way, the endotoxins would not be released. This is where synthetic phages could be particularly suitable.



APPENDIX II.4. INTERVIEW OSCAR KUIPERS

Professor and Head of the Department Molecular Genetics at the University of Groningen.

21-03-2016

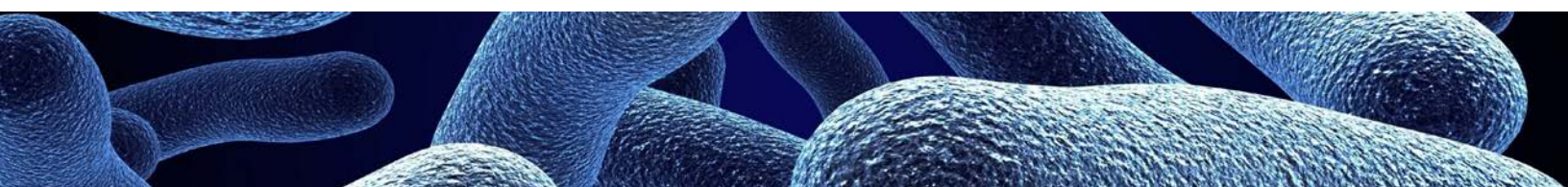
1. *Is it true that a lot of the developments going on in synbio/AMR are still in their proof-of-principle phase? Or are there clinically relevant developments available already?*

   If you are talking about developing new molecules using synbio, I think that that is true. There is relatively few activity regarding filling the pipeline with truly new antibiotics, and even less using synbio. Most developments are still at the beginning of the pipeline, and more time is needed to continue (such as testing of the pharmacokinetics and the clinical trials). We try to do more of these kinds of tests by ourselves as academia so for example executing animal tests, and studying resistance development. Namely, the industry does not want to enter this process too soon, therefore the academia have to execute nearly 60% of the work within this pipeline by themselves. In addition, the business model when it comes to the development of new antibiotics is not good as well. The industry does not make as much money on antiiotics as they do an for example medicines against cancer.

2. *Do you think synbio is a new development?*
   Some people say that synbio is the same meat, but different gravy *(Dutch: oude wijn in nieuwe zakken)*, but I do not completely agree with that. I do think it has a different, new approach. However, it is comprehensive, it is a broad notion. In general, it is expected that synbio will lead to a higher production speed, bigger quantities, new routes, new molecules and a more efficient process.

3. *Does synbio have a lot of potential within AMR in your eyes? Or does it have a higher potential within other applications?*
   I think it can definitely play a role, but it won't be its biggest spin-off. Synbio for example also plays a role in the production of biofuels, platform chemicals, or biosensors. There are many developments, and I were to make a gross estimate, drug development (in general) would only be 25% of this. Making new antimicrobials perhaps 5%. Making antibiotics is not necessarily very difficult, but the high costs, the difficult law and regulations, and the fact that we are used to cheap antibiotics, make it more difficult.

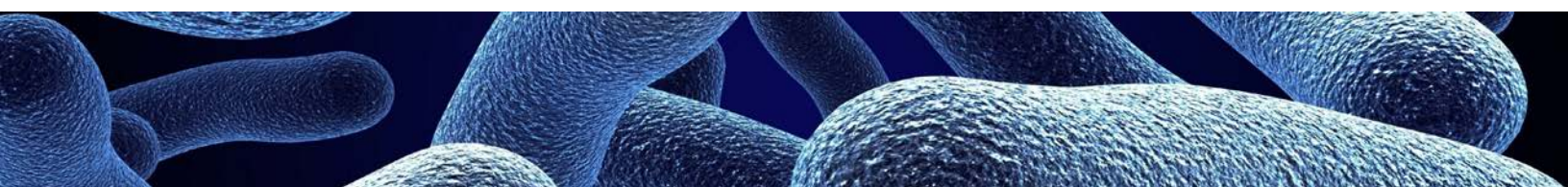
4. *Most developments that I come across are based on DNA. Is work being done using RNA as well?*
   Synthetic biologists often see it as one system, because RNA is derived from DNA. As far as I know, work is being done on this matter, but not as much as on DNA. Using it poses more difficulties I think, because it is single-stranded, compared to double-stranded DNA. It could provide benefits, you could use it for different functionalities, it could function in regulation, or you can use it to create different forms, for example hairpins. Think about aptamers for example, or 'RNA origami'.



5. *Why is it important that synbio is used in the development of lantibiotics? What are the problems that arise during the natural production and use of lantibiotics?*
   With regard to production, the upscaling is the main issue. Here, synthetic biology could play a role in the optimalization of the production pathways. In addition, bacteriocines are not developed in humans, but in the niches of bacteria. Bacteria use it against each other, and they develop resistance mechanisms against them. Definsins are developed in humans, but their evolution has been slower than that of bacteria. Bacteria have a big lead in their evolution compared to humans, they simply exist longer and divide much quicker.
   The main benefit of new-to-nature molecules (developed using synbio) is that there are no resistance mechanisms against them yet. The aim is also to slow down this development. In addition, these molecules might be developed in such a way that they work better in humans. We, and other groups, are combining different bacteriocine modules (for example lanthionines and parts of polytheonamines), in order to create new molecules.

6. How far are the developments within lantibiotics? Are for example collaborations being set up with the industry?
   I think scientists are less naive when it comes to bringing their results to practice, than generally thought. They are definitely open to it. It is true that it is not their most important goal, that is more the main goal of the industry. With regard to lantibiotics, it might be good to test the 'non-synbio' lantibiotics even further. The easiest way to do so it topically, maybe on the skin. The downside of their use in humans, is the fact that the lantibiotic might kill to many bacteria, also the good ones. It is also a large peptide, so it might elicit an immunogenic response. The synbio lantibiotics are not produced on a substantial scale yet, I think that that is the bottleneck. That is why we want to look at the engineering of their productionmechanisms, such as the enzymes that make the lantibiotics. This also takes more time, probably some years.

7. *How far are the developments in using non-canonical amino acids in the lantibiotics?*
   Scientists are not doing very much on this matter. It is possible however, using two methods. In the first method, stop-codons ("amber codons) are placed in a gene sequence, on which a tRNA synthase binds. This synthase holds the non-canonical amino acids, and this way it is placed in the peptide chain. The biggest problem with this is the efficiency/the yield.
   In the second method, auxotrophic strains are used. These are bacterial strains in which the bacteria does not make a certain amino acid, for example tryptophan. They are grown in minimal medium, without the specific amino acid, or the amino acid is removed later on. Next, an analogue of the tryptophan is added to the medium, causing the analogue to take over the place of the tryptophan. The biggest downside in using this method is that the amount of analogues is low, and many mistakes can occur.



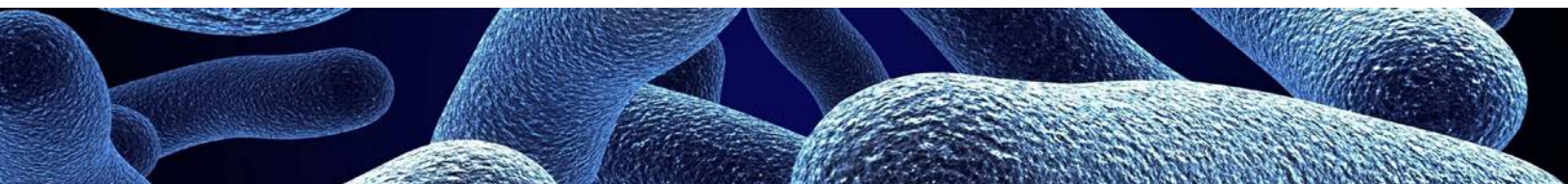
APPENDIX II.5. INTERVIEW MARC BONTEN

Head of the department Medical Microbiology at the UMC Utrecht. One of the initiators of the Netherlands Centre for One Health, scientific employee at the RIVM. 11-02-2016

1. *Is innovation on the research agenda of the NCOH? What is the role of synthetic biology herein?*
   Synthetic biology is not explicitly on the research agenda of the NCOH. However, one of the solution sets is new antibiotics and new therapeutics. The cooperation at the NCOH herein is between the University of Utrecht, the University of Leiden and the Fungal Biodiversity Center. They almost exclusively work on living organisms.

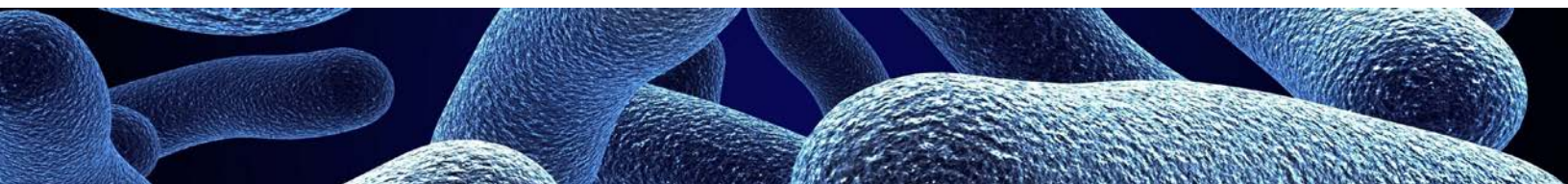
2. *Where is there a lack of matching supply and demand within AMR in the Netherlands? (For example, is there primarily a need for fast diagnostics?)*
   New antibiotics need to be created with a new working mechanism. There are currently nine classes of antibiotics; a tenth needs to be added. New diagnostics are less important. Doctors can, through anamnesis and physical examination, make a good estimate of what antibiotics to prescribe. Diagnostics also exist with which the presence of MRSA (Cephaid) can be tested in 20 minutes. Therefore, there is not that great of a necessity for new diagnostics in hospitals. Perhaps only diagnostics to test whether or not it is a bacterial infection. Concerning the question of diagnostics in e.g. nursing homes, this is more an issue of money and reimbursements. The system in place barely or very limitedly reimburses diagnostics. The knowledge on infection prevention is reasonable and there is a primarily a need for strict guidelines and the following of these guidelines.
   Additionally, the cause of resistance is not necessarily the presence of bacteria on the surface, but the resistance that arises in patients who receive many antibiotics (those who are chronically ill). Resistance that originates abroad and is “brought” to the Netherlands is another large risk. This is something to look out for when entering the Netherlands.

3. *What are the drivers and barriers (e.g. in the current market) for new (innovative) developments in AMR in the Netherlands?*
   Firstly, the earnings model needs to change. Secondly, a new antibiotic should get on the market faster. It takes a long time to test if a new antibiotic is effective and safe. Currently, this takes 10 to 15 years, but we would rather see this happen in 5 years. Classically speaking two phase III studies need to be conducted, but sometimes a fast track can be given on the basis of observational evidence if the urgency is high enough. On a EU level changes should therefore take place in regulation. The most important stimulant for pharmaceutical companies to invest in new antibiotics is money.



4. *What developments/innovations do you see being put into place in the Netherlands in the near future?*
   I do not have a clear perspective on that, but it does not matter much where it comes from as long as it is effective and safe. Immunotherapy is on the rise, the prophylactic administering of antibodies, so that infections can be prevented.

5. *What is the process in the NCOH like? Where do the financial means go precisely, and how is this decided upon?*
   ZonMw handles the applications. They have put out a Dutch call, making it possible to ask foreign experts to judge and rank the proposals. They have a committee who judges these scientifically; the requests are, amongst other things, tested on predetermined criteria. Afterwards, the project proposals are sent out to foreign experts who will judge and rank them.



APPENDIX II.6. INTERVIEW GERARD DEKKER

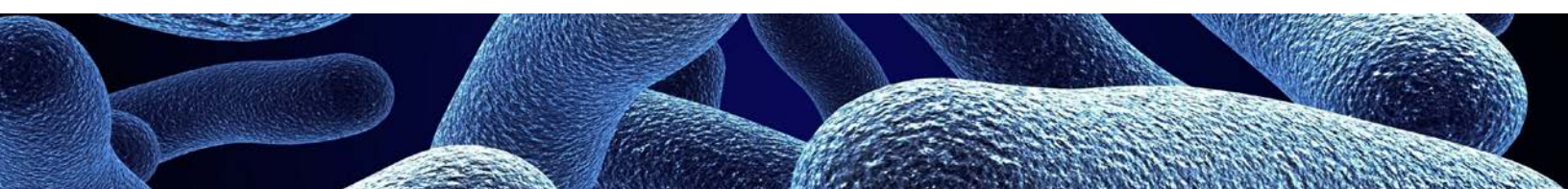
Policy maker, member of the management team long-term care Ministry of Health, Welfare and Sport (VWS). 22-03-2016

1. *VWS has given ZonMw the assignment to create a research program AMR. How did that process go?*
   This research program is the second ZonMw research program AMR. I was not involved in the first research program, but we have gone through its evaluations. The first program was very much focused on drug development. In my opinion it was not very realistic to expect all the solutions in that, when such a complex issue like antibiotic resistance is at hand. Additionally, the varieties that can be created based on existing antibiotics are running out. There is also no real prediction to be made about the chance of gaining new antibiotics. That is why, in this research program, we want to give more attention to other factors. Antibiotics are of course in place to help the body defeat an infection when a person's own immune system cannot handle it. Before someone gets an infection, other factors come into play already, like infection prevention and hygiene. Another factor to be looked at is the (mis)use of antibiotics and its unnecessary prescription.

2. *Are these plans tested for their practical relevance?*
   Amongst the aforementioned issues like infection prevention, but also surveillance, are present in the plans concerning the prevention of antibiotic resistance. Amongst other things, these ideas are tested by several experts. ZonMw ensures that these get discussed with the program team of the previous research program. We do not do that ourselves, it is the responsibility of ZonMw. VWS sees to it that enough feedback is asked and that plenty of other opinions are heard.

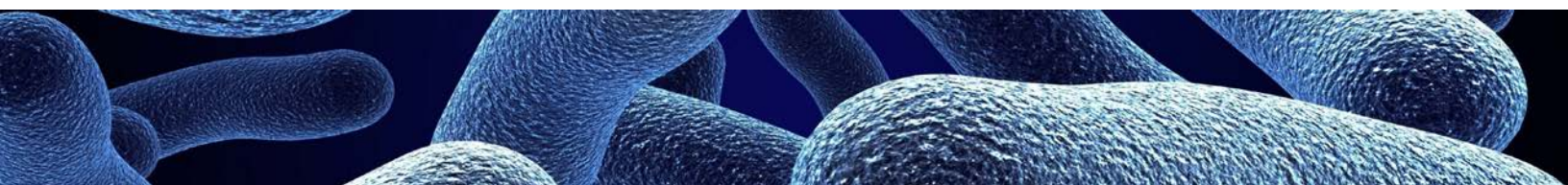
3. *To what extent has this research program been adjusted to the knowledge and knowhow in other countries?*
   Scientific research is already international. Concerning the prevention of antibiotic resistance, the Netherlands is leading. For almost all areas, the Dutch research groups in AMR are in the European top 5. What happens in other countries is still looked at, however. ZonMw looks at Europe's programs when creating the research program. Talking to researchers and experts in the Netherlands makes it internationally oriented already, too, since they keep many international contacts.

4. *What kind of barriers do you see in the development of new antibiotics or alternatives to antibiotics?*
   The restraint that is needed when using new antibiotics means that after development, it will not be used on a large scale. This is a substantial barrier for a successful business case of antibiotics. It is not attractive for companies to focus on developing new antibiotics, especially since the group of people who will need these drugs will be slim (not many patients exist with an infection by a certain multi-resistant bacteria). This problem also occurs in orphan drugs, these drugs also cannot be produced on a large scale, since only a small number of patients need it.



Additionally, it is asked of manufacturers to leave the antibiotic on the shelf for as long as possible.

5. *Does VWS have a facilitating role concerning the contacts between industry and academia?* We, the ones who are concerned with the care-part in the program antibiotic resistance, do not necessarily keep in touch with that purpose. This is more in the area of the Management GMT (Medicines and Medical Technology; *Dutch: Geneesmiddelen en Medische Technologie).* Regarding antibiotic resistance, two interregional projects were set up by the Ministry of Economic Affairs; one in the North of the Netherlands and one between the Netherlands, Belgium and Germany. This enlarges mutual cooperation, including in the area of innovation.



APPENDIX II.7. INTERVIEW MARNIX MEDEMA

Assistant professor Bioinformatics at Wageningen University. 12-05-2016

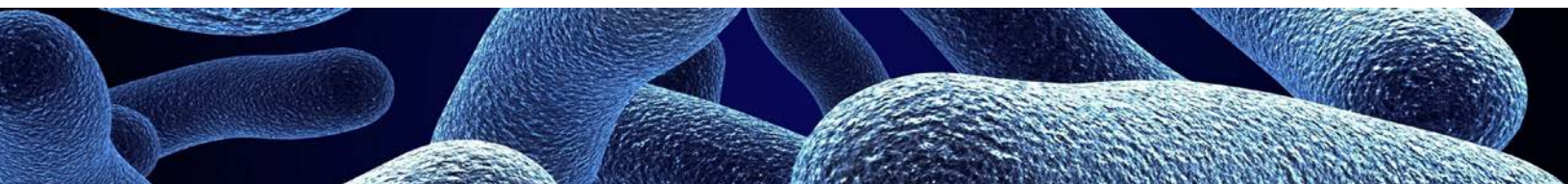
1. *Profile and research*

   Assistant professor Bioinformatics at the University of Wageningen.

   a. *What kind of research do you conduct?*
      My research focuses on the development and application of computational methods to find biosynthetic routes, and this way, new molecules. I focus mostly on bioactive molecules from bacteria that live together with plants, the molecules that plants produce, or molecules from fungi. Partners then use these methods to conduct the experimental work.
   b. *How successful is this?*
      Before, collections of bacterial strains were screened by growing them, and observing if they produced certain molecules. This way, only a small part of the molecules can be found, as not all bacteria produce all molecules that are possible according to their genes under those conditions (on a plate of agar in the lab). Thus, there are so-called 'cryptic' or 'silent' genes that are not expressed under these conditions. Now that we have increased our knowledge on the genome of many bacteria, we can make predictions about which genes encode for the production of bioactive molecules. The biosynthetic routes for certain classes of molecules are easy to predict, although you don't know the structure and activity of a certain molecule. Therefore, we also try to match a certain presence of a gene, or a certain expressions, to certain activities; are there any correlations between them? All of our software is *open source* and can be used by everyone to discover new bioactive molecules.
   c. *Why have you chosen to do this?*
      We believe that publically financed research needs to be publically available. The more it is used, the better.

2. *Synthetic biology*

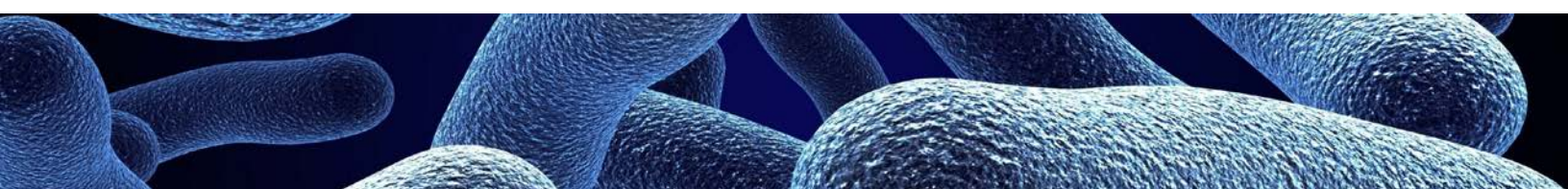
   a. *Do you consider yourself to be a synthetic biologist?*
      Yes and no. On the hand no, because I do not assemble the DNA myself. On the other hand, I am occupied with synthetic biology a lot, and I am developing methods that are being used in synthetic biology. For example, we are currently getting involved with an exciting project from the United States, named '1000 molecules'. In this project we are looking at bacteria that are not cultivated, but for which genetic information is known. This information, which encodes for certain bioactive molecules, can then be reinserted into a host strain, together with its own (synthetic) regulation, after the which the molecules are produced.
   b. *Do you see a lot of potential in synthetic biology in this area?*
      I think it has a lot of potential. Especially when you match it with phenotypical data (for example determining that a lot of bacteria are present in places where almost no diseases occur). Synthetic biology in general will be a 'game-changer' in the next 20 years.



The technology is moving fast, and the first projects are being applied on a large scale as we speak. I think it will have a great impact on the discovery of new natural products. In addition, it will have a lot of impact on the production of chemicals, biofuels, etc. (using bacteria as factories).

c. *To which extent is synthetic biology indeed 'plug-and- play'?*
When it comes to protein-protein interactions for example, we know that it is not a simple 'plug-and-play'. You cannot simply swap part one for part two; the interaction between them is often to refined on a molecular level. You can move a complete pathway from one bacteria to another host. I do think that in the future we will understand the different 'parts' more and more, and it will become 'plug-and-play' more.

3. *Chances and barriers of synthetic biology in the Netherlands.*
   a. *Do you see great chances for synthetic biology in the Netherlands?*
   I think we will have to look at international cooperation mostly, for example as I am doing in projects with the US. A lot of pioneering work has been done there, and they have large-scale facilities. Tens of millions of euros have been spend, money that we might not have this instant in the Netherlands.
   b. *What kind of barriers do you see for synthetic biology and new antibiotics in the Netherlands?*
   Specifically for antibiotics, it is now not very interesting for companies to adhere to it. Concerning synthetic biology, I think the public opinion on the use of modified bacteria is quite favorable at the moment. On the genetic modification of plants on the other hand, I think people are a bit more apprehensive. Despite the fact that this is a much 'cleaner' way technically than for example the crossing of plants after mutagenesis. I think its mainly an emotional argument, which you cannot simply resolve by providing information.
   c. *Who is important with this according to you?*
   I think scientists have to be more involved with society, and remain in close contact with for example policy makers. Good scientific communication is important too. You will have to talk about your own research before others will. DIY synbio is also a possibility to involve more people with current research.
   d. *Do you think policy makers should guide the research that is being conducted more?*
   I think that is something that is very difficult for them, as they generally lack the substantive knowledge to say which type of research will have the most potential. Moreover, scientists also lobby to guide the cash flow towards their specific research area. Some sort of expert panel, existing out of people from universities and industry, would be more suitable for this (something that is also happening currently, I believe). Too much guidance from the government also will come at the expense of creativity I think.



APPENDIX II.8. INTERVIEW ALEX FRIEDRICH

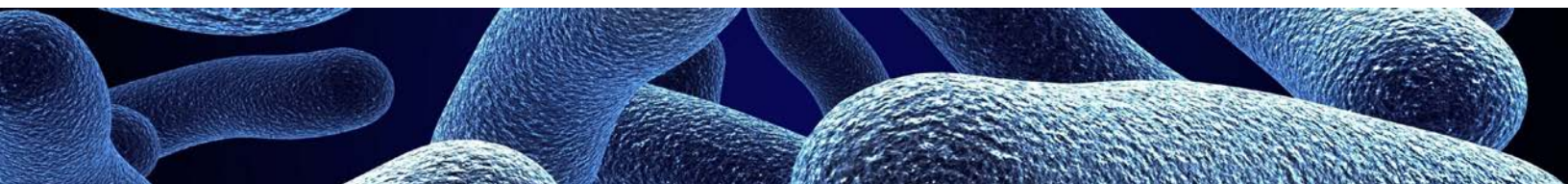
Professor Medical Microbiology at the University of Groningen, Head of the department Medical Microbiology and Infection Prevention at the UMC Groningen. 06-05-2016

1. *Profile*
   a. *Background*
      - Professor Medical Microbiology of the University of Groningen, Head of the Department Medical Microbiology and Infection Prevention of the UMC Groningen.
      - Trained as a medical doctor in Germany, Portugal and Italy. In Germany further trained as a clinical microbiologist, specialized in bacteriology and infection prevention.

   b. *(Active) membership*
      - Board member of the European Society of Clinical Microbiology and Infectious Diseases (ESCMID).
      - Board member of the ESCMID Committee for Infection Control (EUCIC).
      - Board member of the Netherlands Veterinary Medicines Authority (SDa).
      - Member of the Health Council Curative Care.
      - Chair of the national Taskforce 'Medical Microbiology 2025', established by the Dutch Association of Medical Microbiology (NVMM).
      - Former member of the Dutch Working Party on Antibiotic Policy (SWAB).

2. *Indication of the problem from his perspective*
   It is not enough and not sustainably thought to only produce new antibiotics, as we did in the 60s and 70s. The use of an antibiotic has to be an exception, and resistance has to be prevented as much as possible. The prevention of an infection is very important here, and not just the treatment after an infection has occurred. In addition, as a doctor, I would only want to develop antibiotics against the most urgent and important bacteria. The very broad screening for antibiotics therefore does not seem very efficient to me. Moreover, I don't think there is much news to find using the classic method of searching for antibiotics, a much more personalized treatment is necessary.

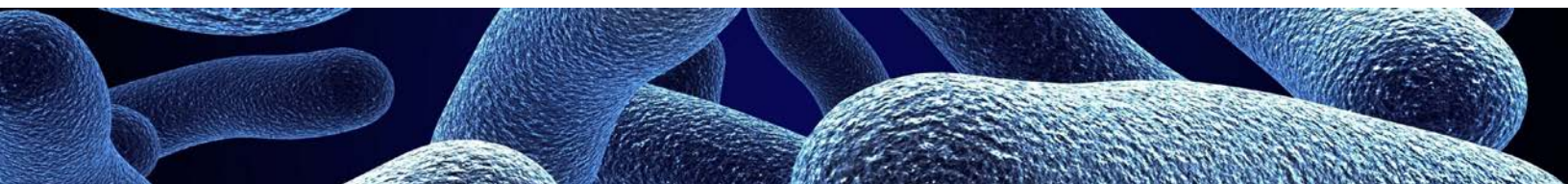
   Differences on national and international level
   Within the Netherlands, there are not many differences between hospitals. In countries outside of the Netherlands, for example in Germany or Greece, there is a difference with regard to infection prevention. Despite this difference with the Netherlands, there is less of a difference between these countries (Germany and Greece). Whereas in Greece this is mainly caused by a lack of financial resources, in Germany this is mainly caused by their prioritization. Namely, there is no direct money to be earned with prevention. For example, this is why, in contrary to the Netherlands, no specialized clinical-microbiologists are active in Germany. In the Netherlands, these functions as the gatekeeper of the quality of diagnostic tests and assure a good translation for infection prevention and the optimal treatment in the hospital and beyond. You cannot just buy clinical microbiologists; it takes years to train them.



3. *Solution directions*
   A lot can be gained in prevention and targeted antibiotic treatment, which specifically and is effective on the right moment and the right place. So: 'smart' antibiotics and targeted drug delivery. For this, appropriate diagnostics are very important. Infections caused by antibiotic resistance bacteria cannot simply be recognized by their symptomatic anymore, no doctor is able to properly diagnose without specific microbiologic diagnostics. Diagnostic stewardship is therefore in the future of great importance in order to properly execute good antibiotic stewardship.
   Barriers
   Right now, diagnostics are not interesting to invest in. While antibiotics are being reimbursed, diagnostics are not. Doctors have to pay for them out of their own budget and indicated diagnostics are being seen as an item of expense and therefore not being applied for. It would be better to offer the diagnostic as 'free', which will be paid as soon as an antibiotic is being prescribed.

4. *Research for new therapeutics and alternatives*
   I think synthetic biology could aid in the development of better diagnostics. Currently, besides using molecular diagnostics, phenotypic diagnostics still have to be used as well. It could be that synbio could enable us in the future to, after determining the genome of the bacterium, to synthesize that genome. This would enable the phenotypic diagnostics to be executed in a much more controlled manner, using for example a built-in apoptosis program, causing the bacterium to inactivate itself.
   Regarding the development of new molecules, I think bacteria will always be able to adapt and develop resistance towards the molecule. We therefore need to understand properly how they do this, so fundamental research remains important. Herein, research is also necessary to investigate how bacteria behave in an organic network, for example in a biofilm.

5. *Whose move is important?*
   I think universities need to play an important role. Policy with participation is necessary, people at the end of the innovation chain need to be involved in the decisions as well. Research programs such as those of ZonMw are very focused on academia and research, but co-creativity is not only possible in research centers. Bigger participation from all directions (for example also from patient organizations and citizen-science) will be important. This is also a way for everyone to recognize the importance of the research that is being conducted. Universities, in addition to the government, have the social responsibility to bring knowledge into society. To obtain 'out-of-the-box' ideas, everybody has to be involved, even though there might be a higher risk associated to it.



An example

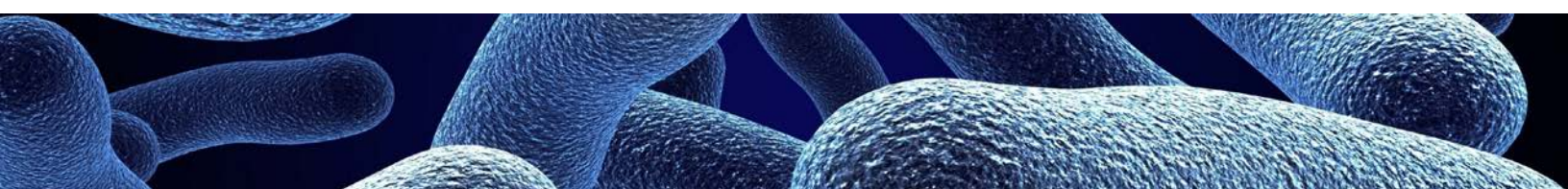
One example of this is the EU-project 'health-i-care', in which science, health and business come together. Already, 30 consortia have started, in which everybody has to comply to three criteria: 1) Contribute to a solution to AMR; 2) Being transboundary with regard to the Dutch and German border regions; 3) Being cross-sectoral with regard to science, business and health, as well as human, animal and the environment.
This way, an 'invisible' glove is being developed; a crème that has an antibacterial working mechanism for several hours. This idea originated because the necessity of using gloves (from a hygienic point of view) was not perceived as pleasant by the end-users (un-personal treatment) in home care and nursery homes.

Technology transfer

One role the government can play in my opinion is stimulating the ongoing developments. Universities are limited in the developing research results further. They simply do not have the resources to produce and globally distribute a new antibiotic. After the university, there are therefore two options: business, or the government. A more sustainable option is a business, but the government can develop things that do not play a role right now, further (something the industry normally does not step in).

A suitable model could therefore be, that the industry at one point takes over from the government, after which they have to pay a certain percentage back to society. We are now focused too much on the division between private and public money, but we should look more at sustainably or non-sustainably aimed. Research should be coming from one big innovation fund, which is being supplemented by industry, health insurance companies and the government. This innovation fund should then also divide this money. This way, businesses and the government share societal responsibility.

This broad approach should also be seen in the financing in AMR and policymaking. The differentiation between more fundamental (NOW) and applied (ZonMw), is less convenient in this subject. In addition, the policy of the different ministries could be more homogenous, as well as that from for example provinces and municipalities, which now also have gotten the responsibility for healthcare. An important aspect of this is good knowledge distribution, so that there will not be too much of an overlap in executing certain projects and *good practices* can be learned from each other.



APPENDIX II.9. INTERVIEW INVESTMENT PROFESSIONAL

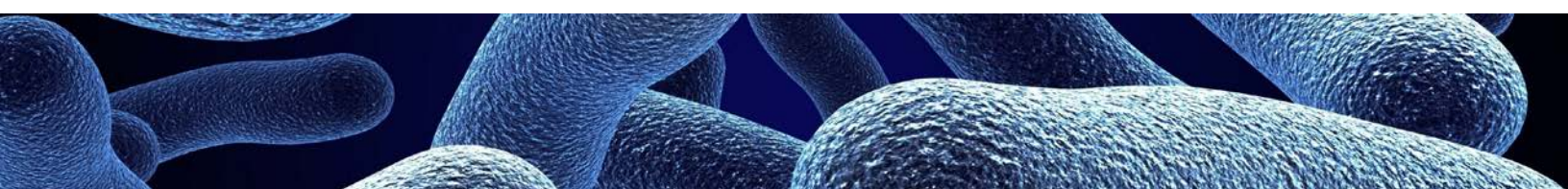
Junior investment professional (associate) at an investment company in the medical sector.

25-05-2016

1. *Profile*

   The company invests in the life science sector, in medical technologies or therapeutics. This is done from a financial point of view, although all employees and investors have affinity with the advancement of care. A difference with a venture capital company that is part of a pharmaceutical company, is the fact that they also invest from a strategic point of view, whereas we only want to create value for our shareholders.
   Among our investors are for example insurance companies, family offices (individuals or families with capital) and in the past sometimes pension funds. These are mostly people or companies that feel connected with the sector. Investments in venture capital over generally more risk baring than other types of investments, because there is a more concentrated portfolio of companies and developments.

2. *Consideration with regard to investment decisions.*
   a. *Where do you get your knowledge from? How do you stay up-to-date? Does this suffice?*
      First off, almost all of our employees have a scientific background, which sets them in the field. In addition, we entrust an elaborate network of experts (so-called opinion leaders), which we have bound to us. On the one hand, they can tell us what is going on, while on the other hand they can advise us about these developments (is it a good investment for us?) We have also established a name for ourselves in the field, which provides us with access to different people, for example if we want to be brought into contact with someone by an expert. We also keep up with documentation, and, because we also have a fund that invests in listed companies, we get information from for example banks that have a research department in that sector. Now and then, when we are relatively unfamiliar with a sector, we ask an expert to compare five or six companies for us, to be able to estimate which company is leading in this field.
   b. *How do you see the innovation chain or pipeline as a whole in general?*
      I think a very general overview of the chain is indeed the route from policy, to financing, to academia, to a business to the end-use. However, there are many variations to this. Some companies are for example taken over after a phase I study, or it is a spin-out of a bigger pharmaceutical company. Additionally, a company can be started with ideas that someone still had (for example from a previous company).
   c. *Do you have a good view on the innovation pipeline? To which extent do you for example keep in contact with knowledge institutions?*
      We have a lot of contact with businesses, so more at the end of the innovation chain. We also take to end-users, such as doctors, if we have to make decisions about investments. We used to go to all knowledge institutions once every three months, but that does not happen anymore.

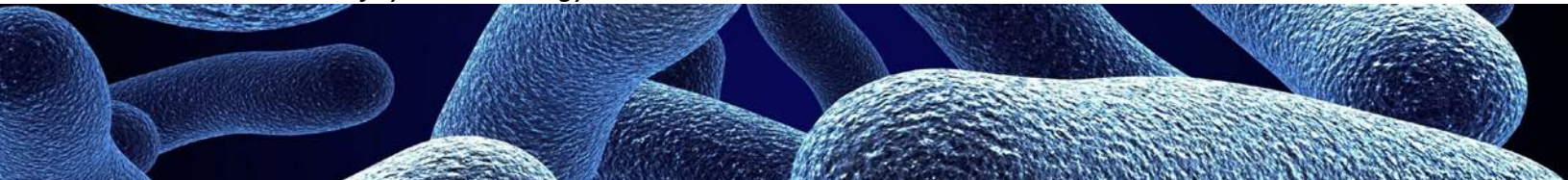


We passively received a lot of propositions, and did not necessarily need to actively search for them. Now that we have more capacity and money, we might start with this again.

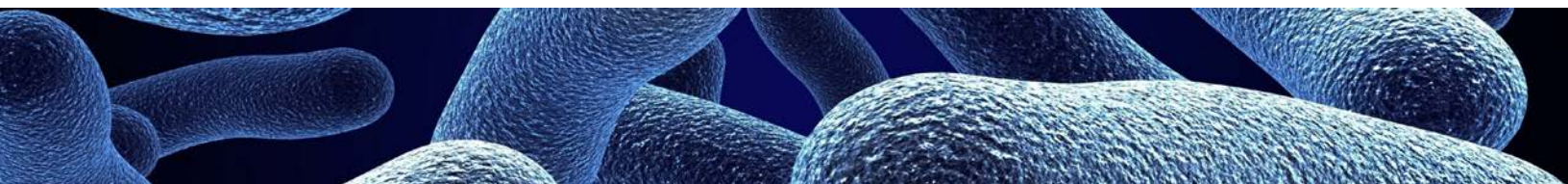
d. *What do you see as the biggest problem in the current approach to develop new therapeutics?*
   In general, if there is a market for it, it will be made. This is not always rational. Let's take diagnostics for example. It is very logical that it needs to be developed and used, everybody would like a personal and argumented treatment, and it can for example lead to better prevention. However, if we receive a proposition from a diagnostics company, there are many obstacles.
   For example, general practitioners or hospitals lack the incentives to buy or use them. I think the barriers are therefore somewhat further in the chain, more economical instead of scientific.
e. *Which considerations do you make when investing in new antibiotics, alternatives or diagnostics?*
   Eventually, it always comes down to who will use the product, if it used in anyway. Need is not necessarily equal to the actual use. There always has to be a financial return. I do see more propositions for antibiotics lately, so maybe the market is picking up. Furthermore, we look at the results of preclinical studies, and how they are planning to do their following studies, how the team is organized that is doing the proposition (do they for example already have experience commercializing products?).
f. *So, in general, you usually step in late in the process?*
   Not necessarily, sometimes we also step in when a development is still early in the innovation chain. We need to have a lot of trust in the team then.

3. *Whose next move is important?*
   a. *Who do you think is to move with regard to the development of new antibiotics or alternatives?*
      I think the government, and more specifically concerning economic incentives. A small incentive can be enough, look for example at the Orphan Drug Act (for orphan drugs). Since this act has been adopted, many drugs for orphan diseases have been developed. There were small incentives, but with a large effect.
   b. *How do you see the role of other in the innovation chain (scientists, SMEs, big pharma, etc.)?*
      I believe strongly in free market processes. I think a lot of knowledge for the development of new therapeutics is already present, but companies or people are not financed. This is due to the fact that there are no financial incentives to do so.
   c. *How do you see the role of the government in stimulating and guiding these developments?*
      That is difficult, on the hand you have to choose where to spend your money one, while on the other hand you have no idea if it will actually work.

4. *The role of synthetic biology*



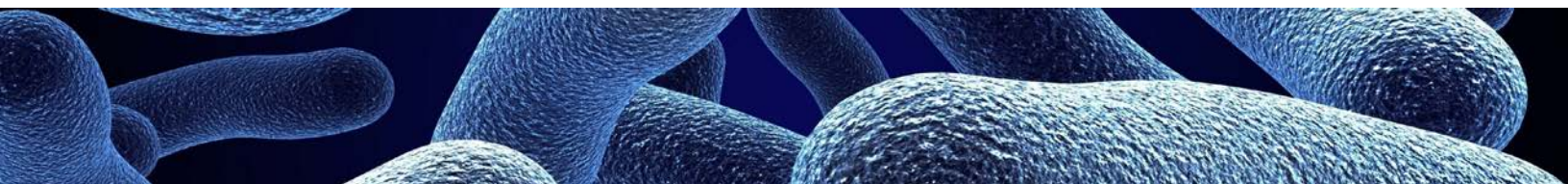
a. *Were you familiar with synthetic biology before the stakeholderdialogue of the Rathenau Institute?*
   Yes, although I still think it is a unclear definition. I knew synthetic biology predominantly in a general sense, maybe not specifically all the developments within AMR.
b. *Which chances and barriers do you see (from your perspective) for these developments?*
   I did not see synthetic biology as the most obvious possibility from which certain developments would come. However, a lot has gone right within this field lately. For us, chances are mainly determined by the actual use of the product by people. This could provide barriers for synbio, although I think the acceptance of the use of genetically modified organisms is much better, and this will not be the biggest barriers anymore.



APPENDIX II.10. INTERVIEW LAURENS LANDEWEERD

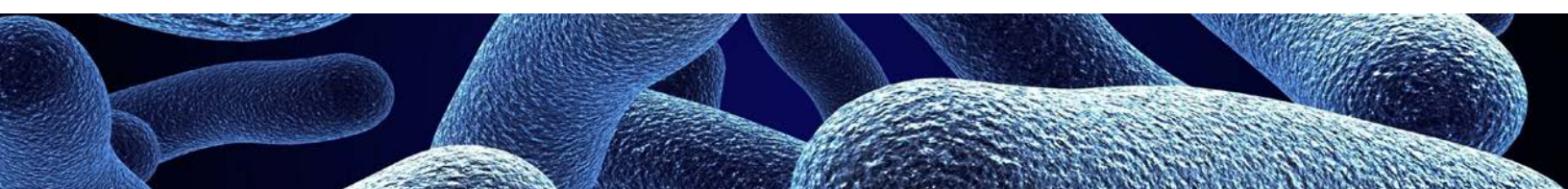
Assistant professor at the Institute for Science Innovation and Society of the Radboud University of Nijmegen. 20-05-2016

1. *Profile*
   Studied philosophy and completed his PhD at the University of Delft (on personal identity in reproductive genetics). He concerns himself with the ethical issues regarding the discussion of new scientific developments. He was involved in the project SyntEthics, in which the ethics of non-medical applications of synthetic biology were discussed. He is also involved in the project SYNENERGENE, which he got for the university of Delft and for which he is also a second promotor of Matti Sonck, the PhD student working on this project.
2. *Ethical issues in synthetic biology*
   a. *Which ethical issues are generally involved in synthetic biology?*
      This is mostly the 'standard' list: safety, a righteous division of the harms and benefits and legislation and patentability (so: who has access to these technologies). With the public this is for example the naturalness of a development, or the moral inviolability of life. These are often issues that scientists are often concerned with to a lesser extent.
   b. *To what extent are these new ethical issues? Or were these already present with for example GMOs?*
      These issues were present then as well. The emphasis now is mainly on the patentability of synthetic biology, which lies on the boundary between discoveries and developments. The question here is mainly if the patentability regimes are still appropriate. Should for example the standardized 'building blocks' become *open access*? In addition, the developments are in my opinion not radically different than the things that were here before, they have built on those developments. It just received a different label. This also creates discussion about the label itself. Synbio scientists, in general, but also within AMR, should be careful not to get involved in these problem discussions too much.
   c. *Are these ethical issues different for the use of synthetic biology within the medical world?*
      Absolutely. People are often critical when it comes to synbio (or GMOs) in food, but in their use in for example the industry (for making biofuels for example) it is often not an issue. They object even less at their use in medication. I could imagine there being a fear against the use of for example engineered bacteria as a drug. Therefore, clinical trials and creating safety mechanisms during development remain very important.

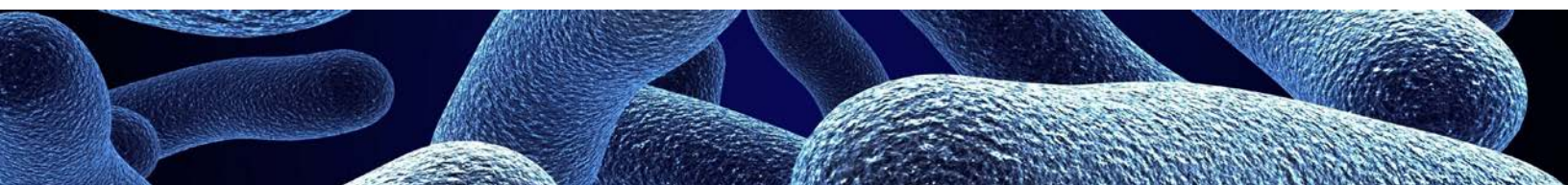


d. *Do these issues differ between certain groups within the innovation chain (scientists, policymakers, industry, end-users)?*
Yes, as said, public ethical issues, such as the naturalness of a development are not as important for scientists. They are often not very aware of the societal discussions that are present. With 'civilians' often the same issues arise (manufacturability of life, no manipulation of nature). Within industry the biggest problem lies in scientific communication. They adapt this to their goal, namely making money. With policymakers, you often see the fact that they are non-experts.

3. *The innovation pipeline or chain*
   a. *Is it a problem that policymakers are generally non-experts?*
   Ignorance is only a problem when there is distrust towards the scientists, something that is seen more and more. A scientists now first has to prove he is an actual expert. Sharing knowledge over the innovation pipeline as a whole does not generate trust in my opinion. You only block up the innovation chain, decreasing spontaneity in research. It might therefore be good that the chain is divided into 'links'.
   b. *Do you think it is a problem that the government often outsources the things for which knowledge is necessary, for example the division of AMR research funding by ZonMw?*
   I think the main problem with that is that the managing of scientific funding is going overboard. Institutions such as NOW or ZonMw feel inclined to restrain from taking responsibilities, by creating long procedures, or using external experts. Not only does this cost a lot of (public) money that could have been spend on research, but these criteria guide the research a lot. This used to be different, but an institutionalized distrust has arisen within scientific funding.
   c. *Why is the guidance of research such a problem?*
   I think guiding or steering research and research results is a fairytale. The best innovations originated spontaneously, or as a byproduct of research. By guiding too much, the spontaneity and creativity of research declines. It would be better if research groups conducting AMR research, would receive a bigger mandate, which can be shown by giving more money to research institutions. Scientists can then divide this money.
   d. *If it might be better that the innovation chain is divided up into parts, what do you think about stimulating the development of public-private partnerships?*
   Upon dividing public money, there is a different type of (moral) duty, than with public money. Innovation routes are often led by wealthy countries, which see the world with a Western view. Upon spending public money, the societal problems of the less wealthy countries are taken into account. When spending private money on the other hand, there are often no societal goals; it is more about the money that can be made.



In public-private partnerships, this dividing line is becoming blurrier, it becomes less obvious where the moral duties lie. This could result in an increase in the following of private goals. This is a process that needs to be guarded constantly. In addition, the ‘revolutions’ often arose from public money, investments made with private money are often much more conservative. By stimulating public-private partnerships, it is promised that the innovation process will be accelerated, but I doubt that. I think it will be better to divide public funding to scientists with a large carte blanche.



APPENDIX III – REPORTS ON MEETINGS

APPENDIX III.1. WORKSHOP: ANTIBIOTIC RESISTANCE AS A SOCIETAL AND SCIENTIFIC CHALLENGE: A ROLE FOR SYNTHETIC BIOLOGY?

On March 23rd and 24th , the Rathenau Insitute organized a stakeholder dialogue on AMR and synthetic biology. The Rathenau Institute functions as the Dutch office for technology assessment and science system assessment. In this role, it contributes to societal debate and the formation of political opinion on issues that relate to, or are the consequence of scientific and technological development. An emphasis lies on the ethical, social, cultural and legal aspects of these developments [231]. Prior to this workshop, I discussed the detailed content of this workshop with the Rathenau Institute, in several meetings.

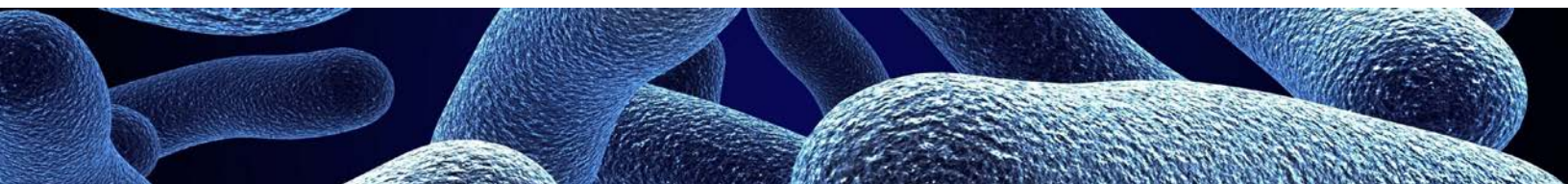
The workshop was held as part of the European SYNENERGENE project, which aims to contribute to Responsible Research and Innovation (RRI) in synthetic biology [232]. The goal of the workshop was to generate ideas about possible contributions of synbio as an answer to the challenges posed by AMR.

The first part of the workshop was an exploration of possible ways for solving AMR and all issues that come along with these possible solutions. Several 'homogenous' groups were formed as much as possible, based their experience with AMR (for example scientists, or policymakers) Next, coherent 'future stories' were set up, in which the following aspects were covered:

- Framing of the problem and the current situation: own experiences with AMR, most important factors contributing to AMR, values and interests that are at stake.
- Preferred solutions/directions for change: what are the most important solutions to this problem, how do these relate to the factors mentioned earlier?
- Which steps are necessary in order develop these solutions: what knowledge, resources and circumstances are needed?

Feedback was then given to these stories by other groups. This included for example the aspects of their story that could be problematic from their point of view, or the way these stories relate to other stories.

The second part of the workshop was focused more on the role of synthetic biology. The synbio scientists and iGEM students gave a short pitch on their research, after which heterogeneous groups were formed. In these groups, the contribution of synthetic biology was discussed to these different future stories. The outcome of this stakeholder dialogue will be published by the Rathenau Institute.



APPENDIX III.2 MIDTERM MEETING

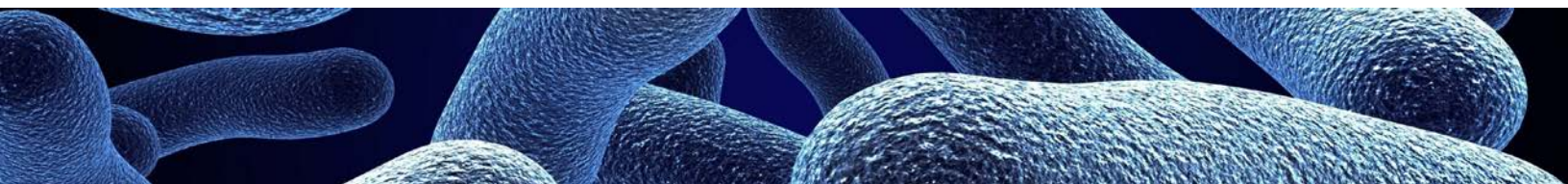
On April 24, I organized a midterm meeting at the RIVM. The goal of this meeting was two-fold: presenting the most important results so far, and receiving input for the remaining part of my internship.

I invited both several people from the CIb as well as from VSP, too create a group with various backgrounds and opinions.

Until that date, I had mainly focused on the 'beta-part' of my internship. An extensive literature study and interviews with synbio scientists had resulted in a listing of the developments within synbio that could contribute to AMR. These I presented, and the q feedback that was given, helped me to identify the weak points in this part.

However, I also had several interviews with other stakeholders and reviewed AMR policy documents. This had led to several ideas about the remaining part of my internship, which would focus more on the integration of these developments with policy. To create a more structured approach, feedback and input was asked from the participants of the meeting. This had led to the focus on seeing the problem from a policy point of view, as where I focused until then on the problem from a scientific point of view. Because the RIVM mainly gives advice towards different ministries, I decided to focus on the role of the government towards innovation in AMR.

In conclusion, this meeting has aided me both in improving the scientific part of my project, as well as the policy part of my project and the integration between both. In addition, both centers were present for this meeting, which can aid them in their cooperation.



APPENDIX III.3. ANTIBIOTICS NOW! SYMPOSIUM

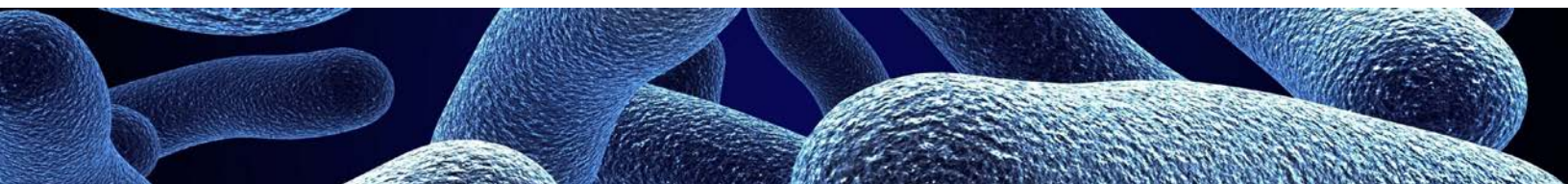
The Antibiotics Now! Symposium was visited for two reasons. First, to obtain information on ongoing research within the field of antibiotics and alternatives to antibiotics. I found it important to know what other developments were going on, in order to put the developments within synthetic biology in perspective. Second, as the visitors of this symposium would be mainly scientists involved in AMR research, or other stakeholders involved in AMR, it provided an excellent opportunity to obtain their perspective on these matters.

This symposium was held in Groningen on the 2nd and 3rd of May 2016, organized by the University of Groningen (RUG) and the Royal Netherlands Academy of Arts and Sciences (KNAW). International experts communicated state-of-the art science from the definition of the problem through the discovery, design and synthesis of new antibiotics as well as the development of alternative approaches.

The program of the symposium (speakers and the subject of their talk):

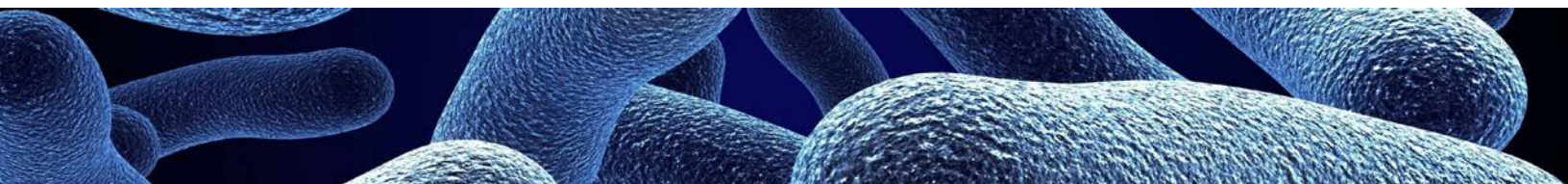
May 2nd, 2016

Time	Speaker	Subject
10.30	Oscar Kuipers (chair)	Welcome
10.40	Edith Schippers	Video message on Dutch initiatives
10.45	Sally Davies	A ticking time bomb: the threat of antimicrobial resistance.
1st session: the resistance problem and why don't we have new drugs?		
11.15	Dirk Brockmann (Berlin)	Measuring the networks that matter in disease dynamics.
11.45	Willem van Schaick (Utrecht)	Antibiotic resistance and the human microbiome.
12.15	Heike Brötz-Oesterhelt (Tuebingen)	Past and present strategies in antibiotic drug discovery.
2nd session: How to open up the pipeline		
14.00	Floris Rutjes (Nijmegen)	Early stage discovery of small molecule antibiotics.
14.30	Rainer Fischer (Aachen)	Facilitating drug development.
15.00	Christina Vandenbroucke-Grauls (Amsterdam)	Drugs and resistance: something old and something new.
3rd session: New leads from the lab		
16.00	Arnold Driessen (Groningen)	Filamentous fungi as resource and production host for bioactive compounds.
16.30	Oscar Kuipers (Groningen)	Novel antimicrobials by synthetic biology.
17.00	Nathanial Martin (Utrecht)	New antibiotic strategies: targeting the lipid II pathway from start to finish.
17.30	Jasper Knoester (Groningen)	



May 3rd, 2016

Time	Speaker	Subject
4th session: New candidates from nature.		
09.00	Anna Vagstad (Zürich)	Proteusin peptide natural products, an expanding repertoire of post-translational modifications.
09.30	Gilles van Wezel (Leiden)	Biological insights as basis for the discovery of novel antibiotics.
10.00	Bob Hancock (Vancouver)	Alternatives to antibiotics- new approaches to treating recalcitrant infections.
5th session: Synthesis and modification of antibiotics		
11.00	Ben Feringa (Groningen)	Photopharmacology: light on antibiotics.
11.30	Florian Kloss (Jena)	Neglected bacteria as prolific sources of unprecedented antibiotic scaffolds.
12.00	Adri Minnaard (Groningen)	Antibiotics against *Mycobacterium tuberculosis.*
6th session: Other viewpoints		
13.30	Henri Verbrugh (Rotterdam)	Phage therapy, now or never…?
14.00	Maite Muniesa Perez (Barcelona)	Phage-related horizontal transfer of resistance.
14.30	Jan van Hest (Nijmegen)	Novel approaches to targeted drug delivery.
7th session: The whole chain		
15.30	Ursula Theuretzbacher (Vienna)	New economic models for antibiotic R&D.
16.00	Bhanu Sinha (Groningen)	An integrated view of the entire chain for development.
16.30	Jos van der Meer (Nijmegen)	Closing remarks.



APPENDIX IV – QUESTIONNAIRE

IV.1. QUESTIONNAIRE ON NEW ANTIBIOTICS AND ALTERNATIVES TO ANTIBIOTICS

The aim of the questionnaire is to gather the opinion of scientists (and other stakeholders) on the viability of the current developments of new antibiotics and alternatives to antibiotics. It is part of my graduate research of the master Biomedical Sciences. All information will be processed anonymously and confidentially.

I. A few background questions:

1. **What is your profession?**

2. **In the case of conducting research yourself, what is your area of expertise?**

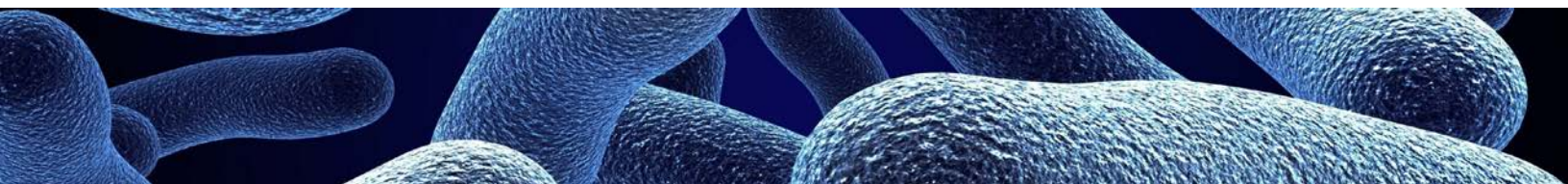
3. **What do you consider yourself to be with regard to your knowledge on developments within the field of antibiotics and alternatives to antibiotics?** *Please circle your answer.*

   Novice – Advanced beginner – Competent – Proficient – Expert

II. Questions on new antibiotics and alternatives

1. **Suppose you have a large sum of money to stimulate any development that would provide society with new antibiotics or an alternative to antibiotics, what would you spend the money on?**

2. **What is the reasoning behind this choice?**



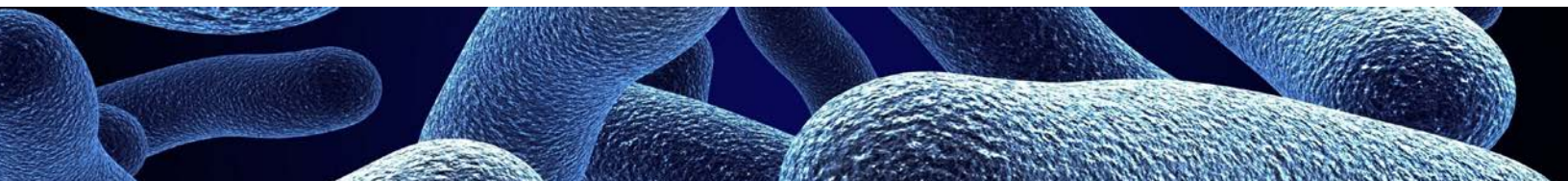
3. **Can you create a <u>top 3</u> of the following developments from highest potential (defined as being able to reach the market the fastest) to lowest potential in providing society with new antibiotics or alternatives to antibiotics?**
   (1 defined as having the highest potential and 3 defined as having the lowest potential. <u>Fill in 1 to 3 in the boxes before the given options.</u>)

	Phage therapy: natural or engineered viruses that attack and kill bacteria.
	Lysins: Enzymes that directly and quickly act on bacteria.
	Antibodies: Proteins that bind to particular bacteria or their products, restricting their ability to cause disease.
	Probiotics: Living micro-organisms that prevent pathogenic bacteria colonizing the gut.
	Immune stimulation: Boosts the patient's natural immune system.
	Peptides: Innate immunity; bacteriocins.
	New-to-nature molecules: molecules that do not exist in nature (either peptides or small molecules).
	Modifications of existing natural compounds.
	Mining and exploring natural sources.
	Other:….

4. **What, according to you, is/are the most important aspect(s) or considerations for being a development with the 'highest potential'?**

5. **What is, from a scientific point of view, generally needed (<u>besides</u> money) in order to help the developments mentioned in questions 1 and 3?**

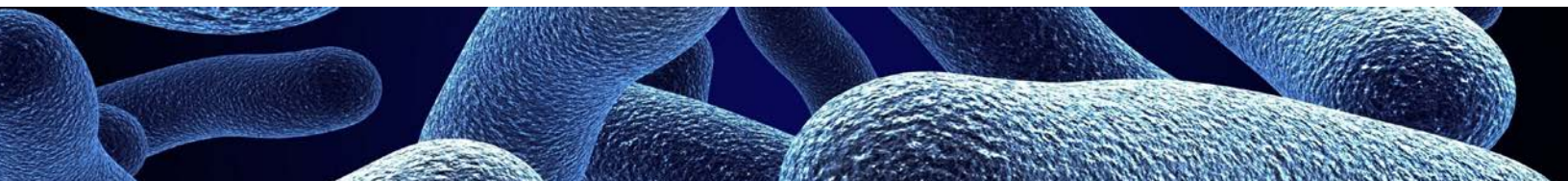
	Not important at all				*Very important*
Scientific education:	O	O	O	O	O
Infrastructure for research (e.g. facilities):	O	O	O	O	O
More public-private partnerships:	O	O	O	O	O
Simplifying drug approval	O	O	O	O	O
More scientific cooperation (networks)	O	O	O	O	O
Public dialogue	O	O	O	O	O
Other:…	O	O	O	O	O



6. Whose next move will be most important to advance developments?

	Not important at all				*Very*
Government	O	O	O	O	O
Big pharmaceutical companies	O	O	O	O	O
Small and medium enterprises (SMEs)	O	O	O	O	O
Knowledge institutions	O	O	O	O	O
Investment companies	O	O	O	O	O
Other:…	O	O	O	O	O

Do you have any final remarks?



APPENDIX IV.2. OUTCOME OF THE QUESTIONNAIRE

To gather the opinion of scientists (and other stakeholders present) on the viability of the current developments of new antibiotics and alternatives to antibiotics, a questionnaire was held at the Antibiotics Now! Symposium (Appendix III.3). This symposium was held in Groningen on the 2nd and 3rd of May 2016, organized by the University of Groningen (RUG) and the Royal Netherlands Academy of Arts and Sciences (KNAW). International experts communicated state-of-the art science from the definition of the problem through the discovery, design and synthesis of new antibiotics as well as the development of alternative approaches. The complete program can be found in Appendix III.3.

Background of the participants

Of the 100 questionnaires that were handed out to the participants of the symposium, 60 were returned. Of these 60 people, the majority (54 people) were academics, participating in research themselves. Their field of expertise was either (bio) organic chemistry (42 people), or microbiology (14 people).

When asked about their profession, 11 people said to be either a professor or associate professor, and 19 people said to be either a post-doc or PhD student (Figure 34). From 21 people, no specific profession could be deduced from their answer, although it was clear that they were all scientists to some degree.

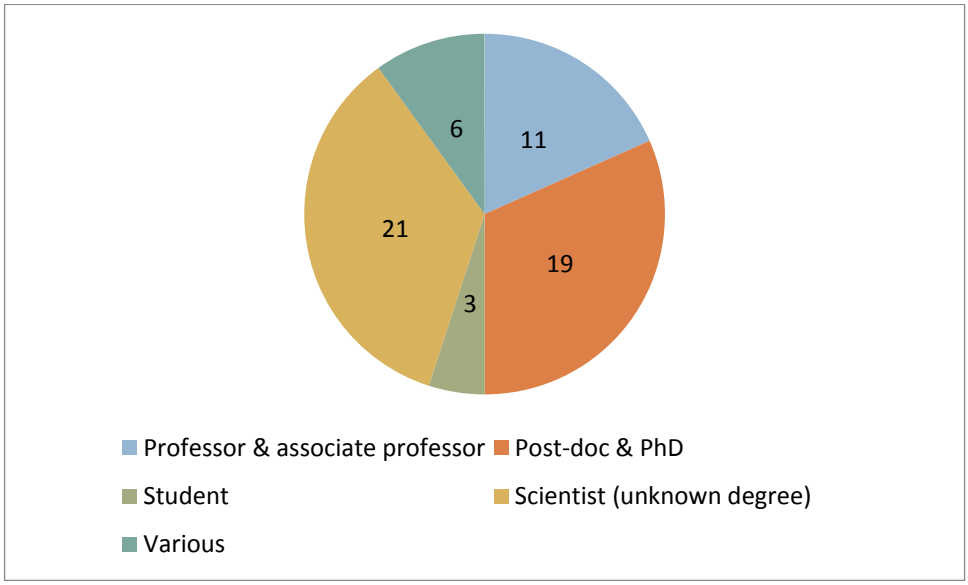
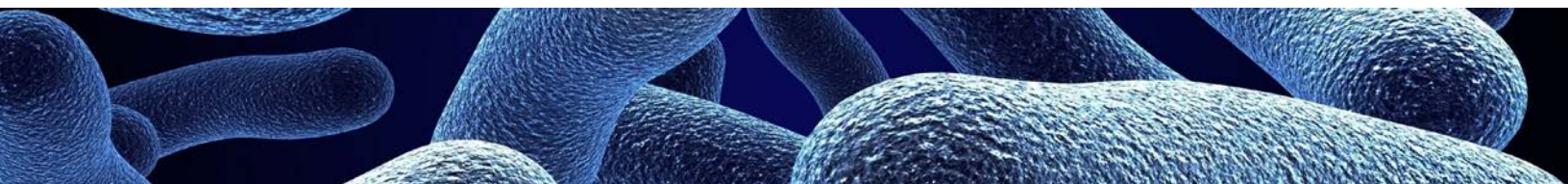


FIGURE 34: PROFESSION OF THE PARTICIPANTS OF THE QUESTIONNAIRE (N=60).

Even more important was their perceived competency with regard to their knowledge on the developments in new antibiotics and alternatives to antibiotics. Therefore, participants were asked to score themselves as being either a novice, an advanced beginner, competent, proficient, or an expert on this subject (Figure 35).



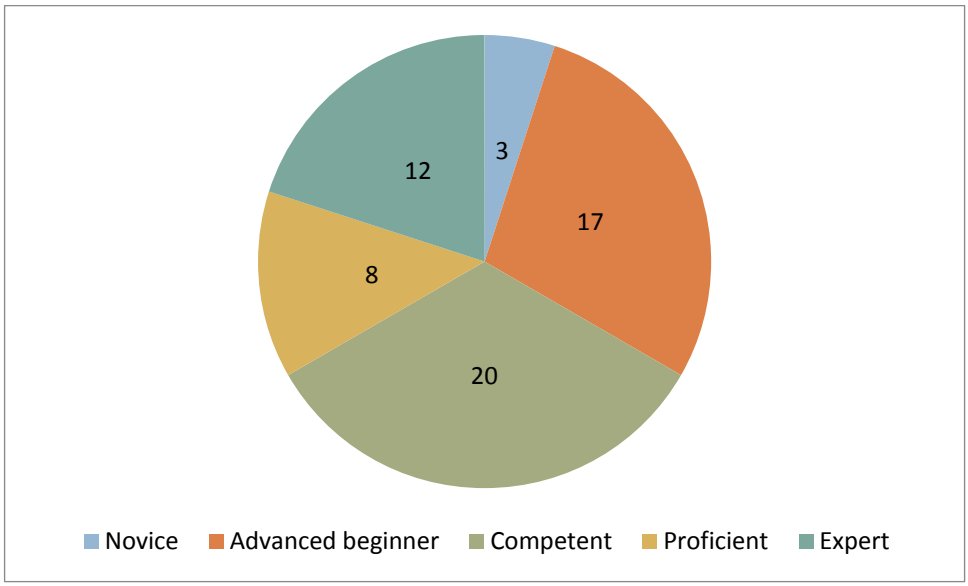


FIGURE 25: PERCEIVED COMPETENCY OF PARTICIPANTS WITH REGARD TO THEIR KNOWLEDGE ON THE DEVELOPMENTS IN NEW ANTIBIOTICS AND ALTERNATIVES TO ANTIBIOTICS.

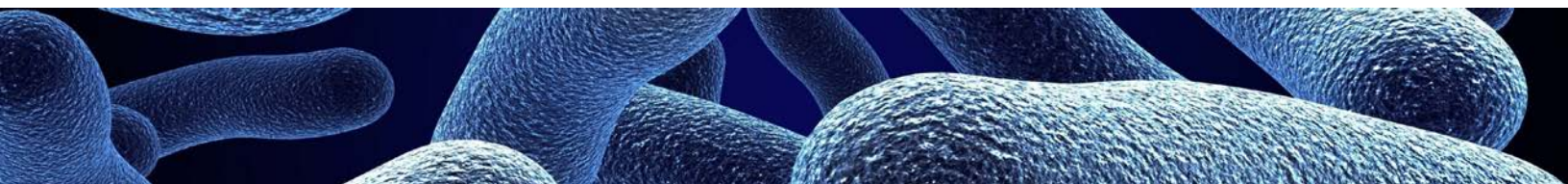
3 people perceived themselves to be a novice on this subject, 17 people as an advanced beginner, 20 people thought they were competent, 8 people proficient, and 12 people perceived themselves to be an expert. To be able to compare the results obtained in the following questions, the results were grouped in equal groups: novice and advanced beginner (n=20), competent (n=20), and proficient and expert (n=20).

Results – Potential of developments

Question 1: First an open question was asked to determine which development people would think would be the one with the most potential, without defining potential and without guiding the question towards a specific development:

"Suppose you have a large sum of money to stimulate any development that would provide society with new antibiotics or an alternative to antibiotics, what would you spend the money on?"

All answers varied greatly, indicating that there is, when openly asked, no one clear development that is seen as the one with the highest potential. Upon categorizing of the answers, many people (n=13) indicated that they would spend the money on the mining/ screening for new antibiotics in nature (Figure 36). Noteworthy is also that 9 people would spend their money on improving research infrastructure, or various other non-scientific measures such as informing the public, and investing in small-and medium enterprises that would bring these developments into production.



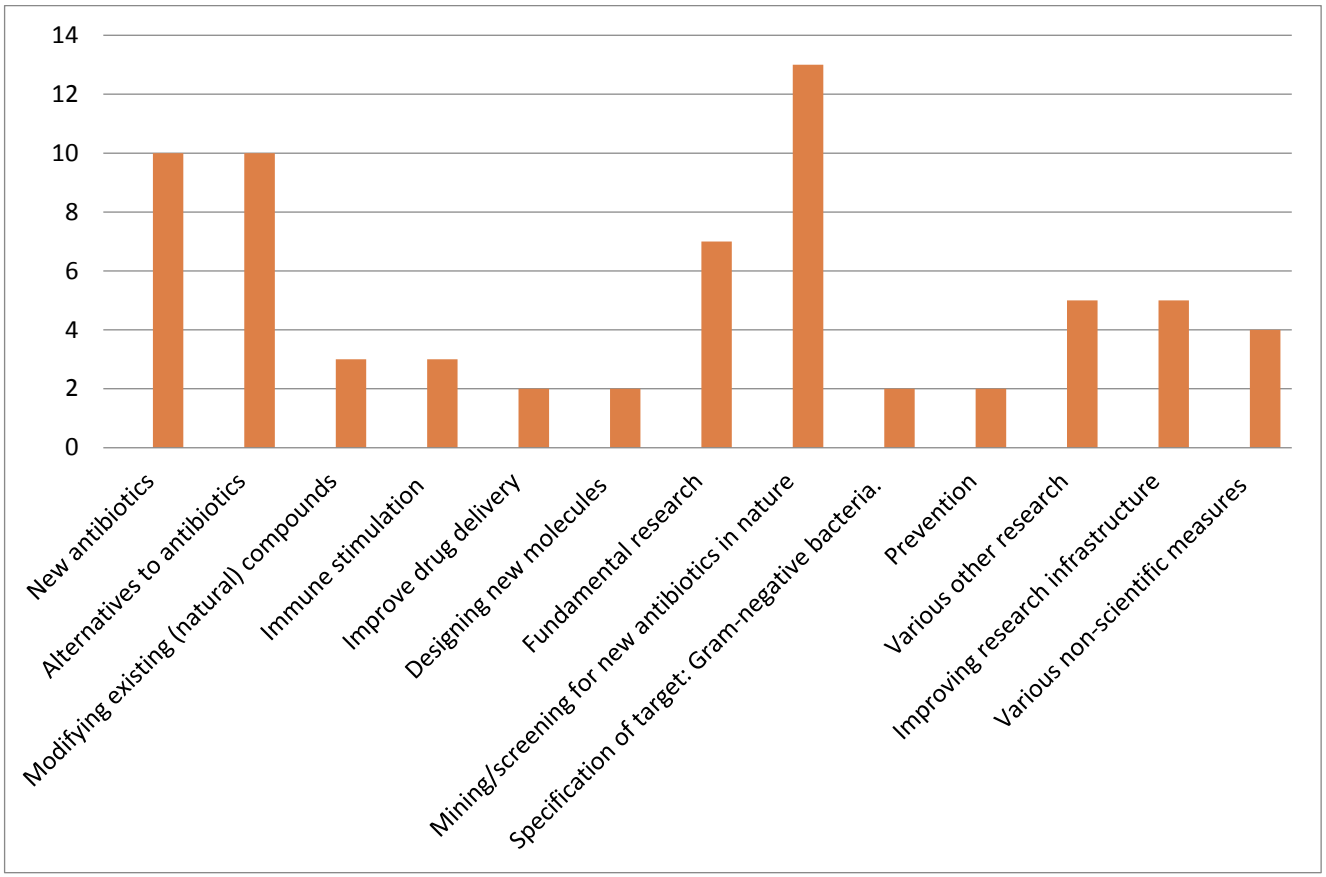
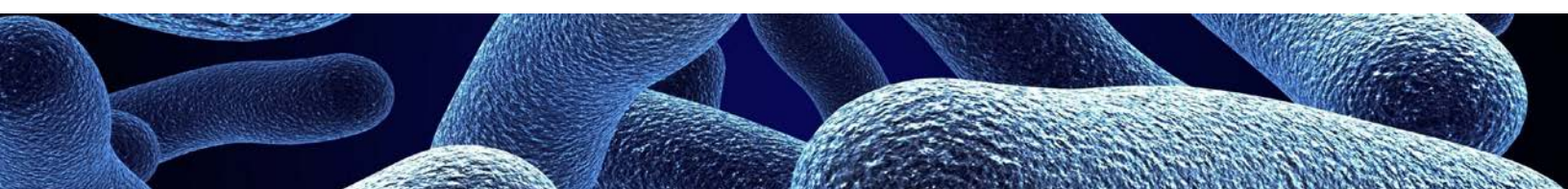


FIGURE 36: SPENDITURE OF A HYPOTHETICAL SUM OF MONEY ON ANY DEVELOPMENT THAT WOULD PROVIDE SOCIETY WITH NEW ANTIBIOTICS OR AN ALTERNATIVE TO ANTIBIOTICS. N=68 (INCLUDING 3 PEOPLE STATING 3 DIFFERENT DEVELOPMENTS, 10 PEOPLE ANSWERING 'NEW ANTIBIOTICS', AND 10 PEOPLE ANSWERING 'ALTERNATIVES TO ANTIBIOTICS').

In addition, it has to be noted that 20 people answered the question with either 'new antibiotics'(n=10) or 'alternatives to antibiotics' (n=10). This is probably due to the framing of the question, which could suggest a choice between both.

Question 2: When asked about the reasoning about their choice for this question, again the answers varied greatly. Mining/screening for new antibiotics in nature was answered for example by someone, because 'it is the only efficient approach so far'. Another participant stated to spend the money on the creation of a dedicated research institute ("European Centre for Antibiotic Development") rather than funding individual research groups or ad hoc networks. The reasoning behind this answer was the fact that "AMR is a multifactorial problem that can only be solved by interdisciplinary collaborations". Within this line of reasoning it was stated by someone that a whole new research framework should be developed, which would be open science based.



Question 3: Next, participants were asked to create a top 3 of 9 selected developments with the highest potential (and one open option, 'other') (Figure 37). 1 defined as having the highest potential, and 3 defined as having the lowest potential of the three. These developments (and definitions) were selected based partly on the report on vaccines and alternative approaches by the O'Neill Commission[98], and the review that has been made in this report. Highest potential was defined here as being able to reach the market the fastest. In other words, the development that would bring society a new product in the tackling of AMR, the fastest.

The 9 selected developments were:

1. Phage therapy: natural or engineered viruses that attack and kill bacteria.
2. Lysins: enzymes directly and quickly act on bacteria.
3. Antibodies: proteins that bind to particular bacteria or their products, restricting their ability to cause disease.
4. Probiotics: Living micro-organisms that prevent pathogenic bacteria colonizing the gut.
5. Immune stimulation: Boosts the patient's natural immune system.
6. Peptides: innate immunity (bacteriocins).
7. New-to-nature molecules: molecules that do not exist in nature (either peptides or small molecules).
8. Modifications to existing natural compounds.
9. Mining and exploring natural sources.

Option 10 could be chosen if an answer was not mentioned in this list. Note: Some participants crossed off three developments, without a specific ranking. In addition, two of them crossed off only two, or as much as four options. This resulted in 29 choices that were grouped into the group 'unranked', for which 1 point per choice was given.

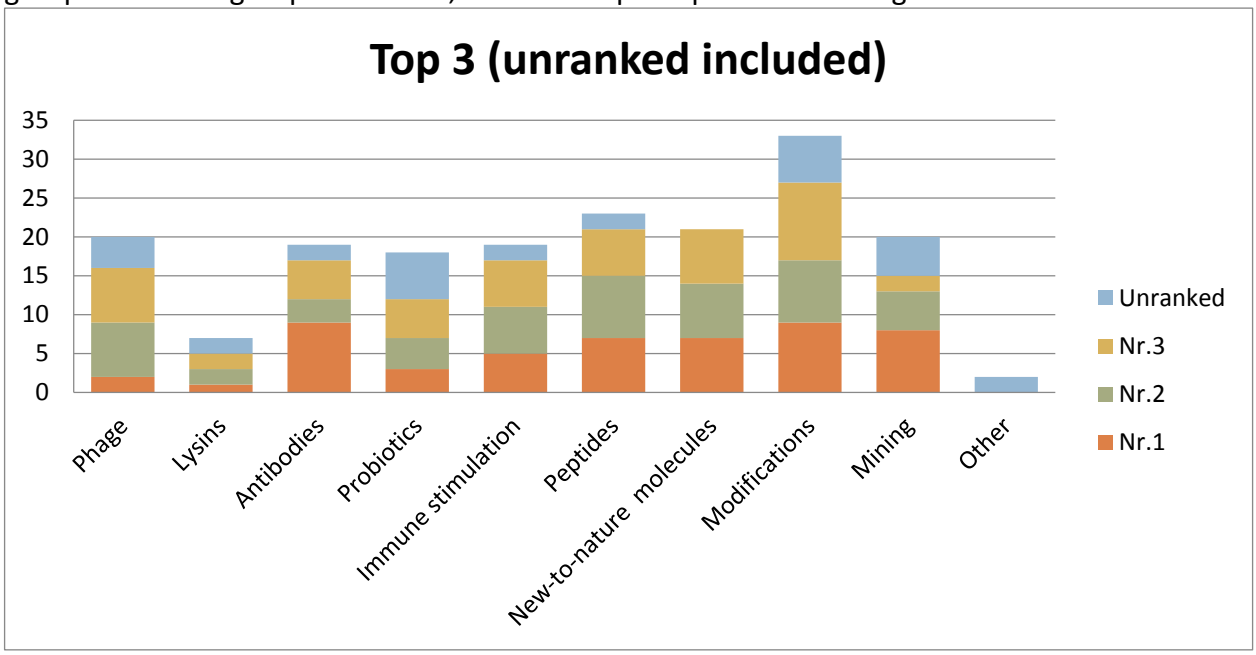
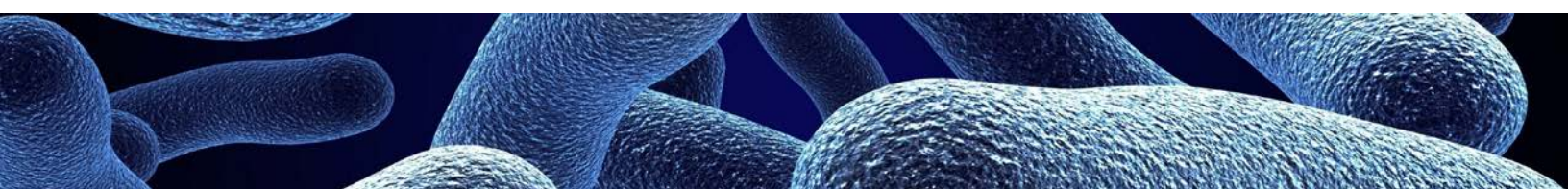


FIGURE 37: NUMBER OF TIMES A DEVELOPMENT WAS CHOSEN AS PART OF A TOP 3 OF DEVELOPMENTS WITH THE HIGHEST POTENTIAL. A TOTAL OF 209 VOTES WAS CASTED BY 60 PARTICIPANTS.



Most people chose making modifications to existing natural compounds to be the development with the highest potential (33 votes) **(Figure 22)**. In contrast, few people thought the same of the use of lysins (7 votes). Only one person in the 'expert and proficient' group chose this option (unranked) to be part of his or hers top 3.

In addition, only two 'other' answers were given. One stated that a different research framework was of importance, the other research on chemical ecology and species interactions. The lack of answers within the 'other' category is also an indication that this list is almost all-encompassing.

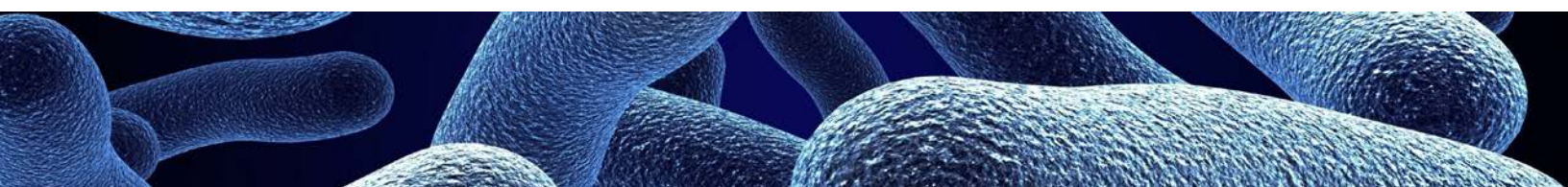
Question 4: As the definition of 'potential' given in question 3 (being able to reach the market the fastest) is only one of the many definitions, the participants were asked how they would interpret it.

"What, according to you, is/are the most important aspect(s) or considerations for being a development with the 'highest potential'?

Again, the variety in the answers was big, making it difficult to categorize them. Most answers focused, not surprisingly for scientists, on various scientific aspects. The two aspects that were mentioned most were an easy (high)-scale production and low toxicity.

Conclusion

There is little agreement on the potential of developments that would lead to new antibiotics or alternatives. However, when asked on which developments people would spend a sum of money, 13 of the 60 participants answered the mining/screening for new antibiotics in nature. This answer was also chosen many times as part of a top 3 of a number of given developments, although many other were as well (a high variety is again seen). 33 of the 60 participants would choose to create modifications to existing compounds as the development with the highest potential. Lysins on the other hand, were in total only chosen 7 times as part of a top 3, indicating that its potential is not widely seen. However, it could be that a different definition of 'potential' would have led to different answers. As mentioned in question 4, this could perhaps be 'an easy (high)-scale production' or 'low toxicity'.



Results- What is needed and who is important?

Question 5: To understand what AMR scientists perceived to be most important to help these developments, the following question was asked:

"What is, from a scientific point of view, generally needed (besides money) in order to help the developments mentioned in question 1 and question 3?"

7 options were given (including one 'other' option), after which these options had to be scored on a five point scale, ranging from 'not important at all' (1) to 'very important'(5).

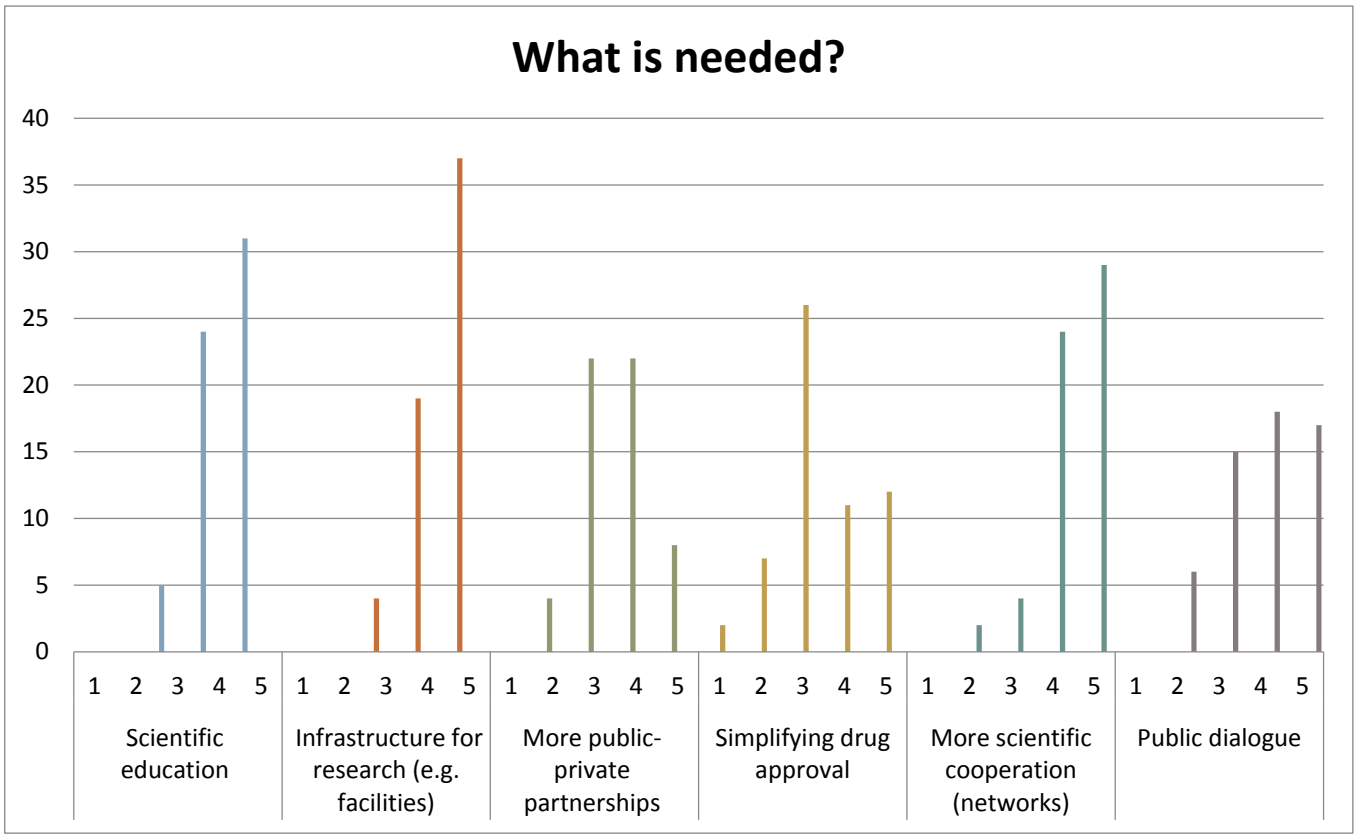
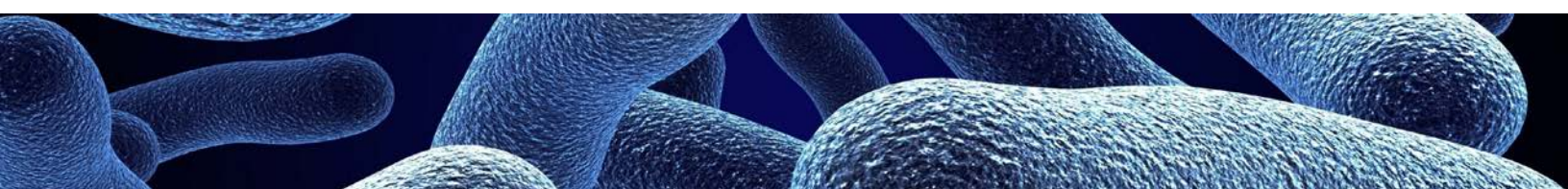
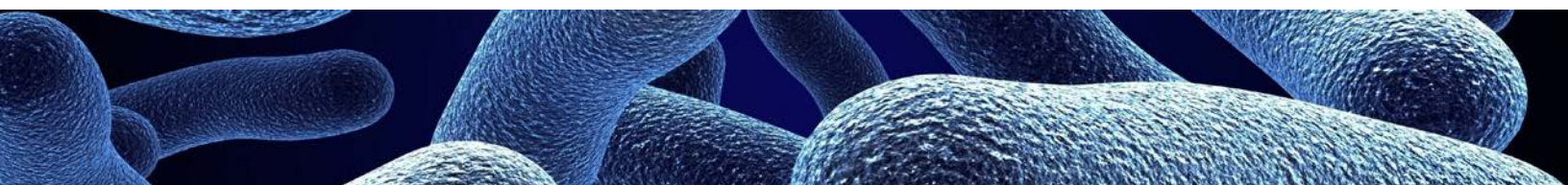


FIGURE 38: WHAT IS, FROM A SCIENTIFIC POINT OF VIEW, IS GENERALLY PERCEIVED TO BE NEEDED IN ORDER TO HELP THE DEVELOPMENTS MENTIONED. RANGE 1-5; 1 BEING NOT IMPORTANT AT ALL, 5 BEING VERY IMPORTANT. FROM LEFT TO RIGHT: N=60; N=60; N=56;N=58



As can be seen in figure 38, ‘scientific education’, ‘infrastructure for research (e.g. facilities)’, and ‘more scientific cooperation (networks)’ were found to be most important. The amount of participants choosing either 4 or 5 for these options is 55, 56 and 53 respectively. As almost all participants conduct scientific research, this is quite logical. ‘More public-private partnerships’, ‘simplifying drug approval’, and ‘public dialogue’ on the other hand, are seen as important as well, but to a lesser degree. The amount of participants choosing either 4 or 5 for these options is 30, 23 and 35 respectively. No clear difference between competency groups can be seen. Other options that were seen as important were ‘more basic research’, ‘trust of funding agencies and governments to use budgets’ and ‘coordination of research focus’.



Question 6: Next, participants were asked whose next move would be most important to advance these developments. 6 options were given (including one 'other' option).

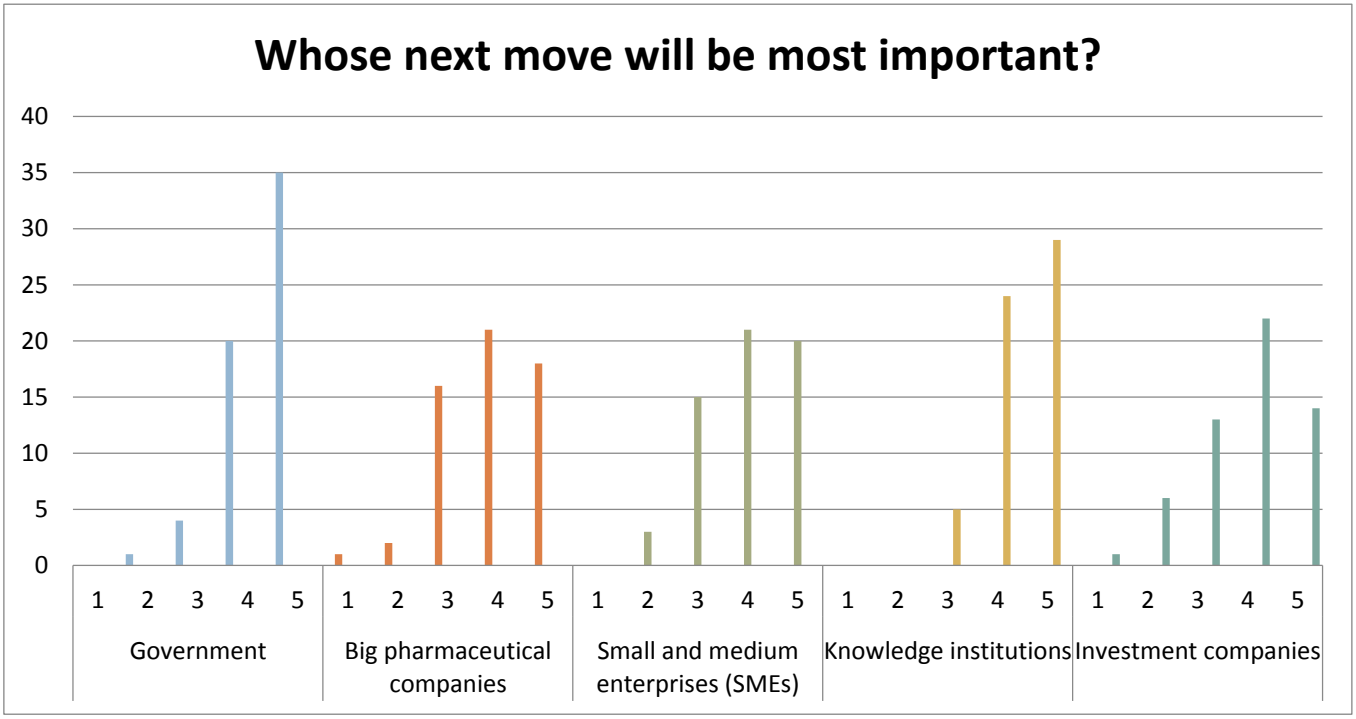
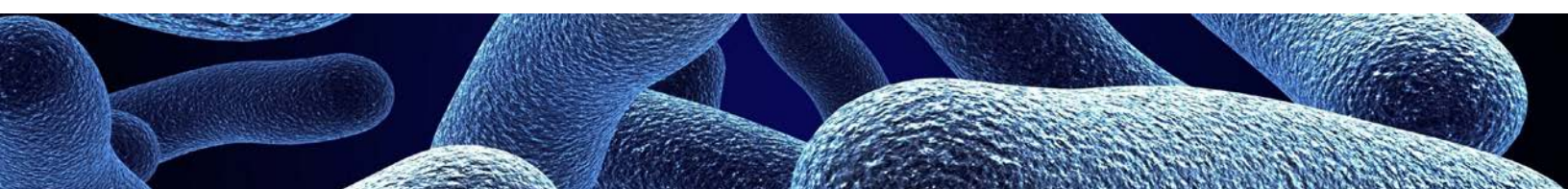


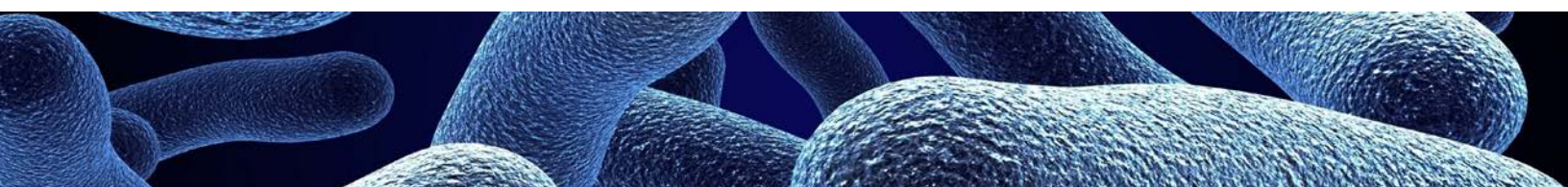
FIGURE 39: WHOSE MOVE IS PERCEIVED TO BE MOST IMPORTANT IN ADVANCING THESE DEVELOPMENTS? RANGE 1-5; 1 BEING NOT IMPORTANT AT ALL, 5 BEING VERY IMPORTANT. FROM LEFT TO RIGHT: N=60;N=58;N=59;N=58;N=56.

As can be seen in figure 39, many participants see the next move of the government and knowledge institutions as very important. 55 and 53 participants chose either 4 or 5 for these options respectively. The role of the government however, can be manifold. Not only can they play a role in solving regulatory issues and financing, but also in creating a better economic model for antibiotic reimbursement.



Conclusion

From a scientific point of view, AMR scientists value interventions focusing on academia and research most. Scientific education, infrastructure for research and more scientific cooperation are perceived to be most important in order to help the developments leading to new antibiotics and alternatives. However, the importance of public-private partnerships, the simplification of drug approval and public dialogue is acknowledged as well. When asked whose next move would be most important to advance these developments, the government and knowledge institutions received the highest scores. Unfortunately, due to time constraints, it was not asked what this next move would have to be. For knowledge institutions, it could be that more specific research and education should be focused on the development of new antibiotics and alternatives. Governmental interventions can include addressing regulatory issues (such as simplifying drug approval or restricting antibiotic use), an increase in research funding, or improving the economic model for antibiotic reimbursement. However, addressing regulatory issues that focus on simplifying drug approval is from a scientific point of view generally not seen as very important.



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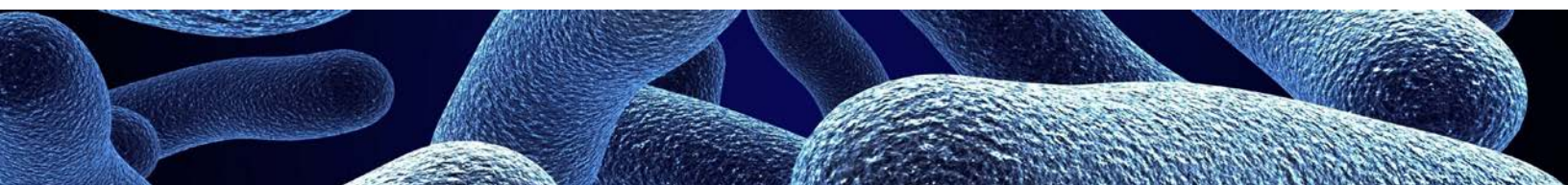

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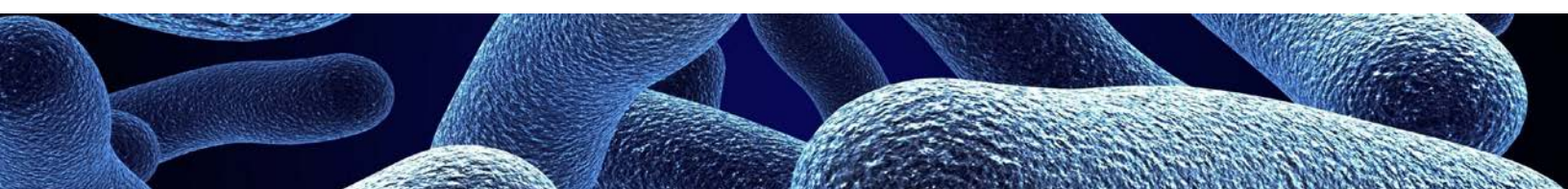

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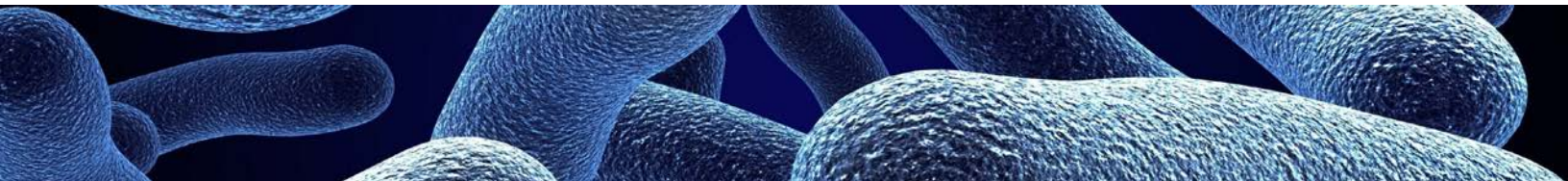

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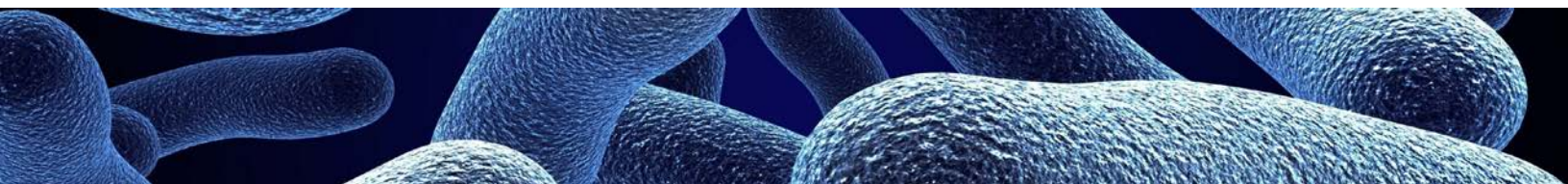

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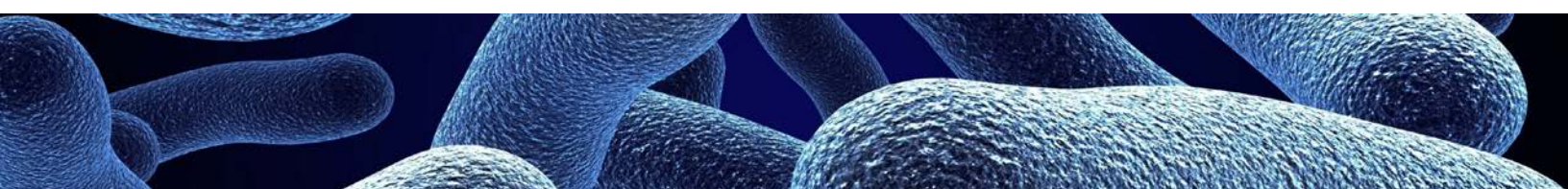

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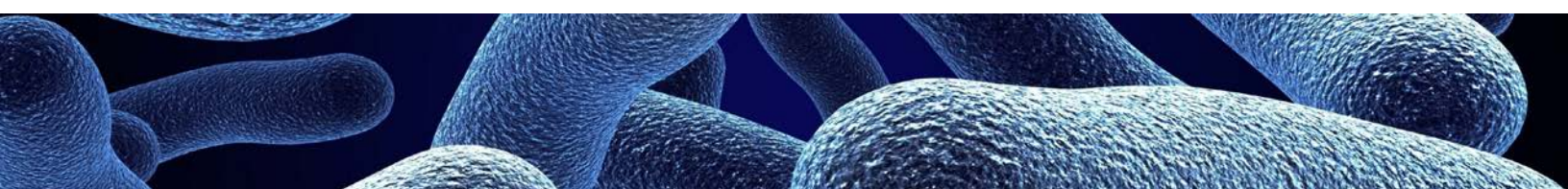

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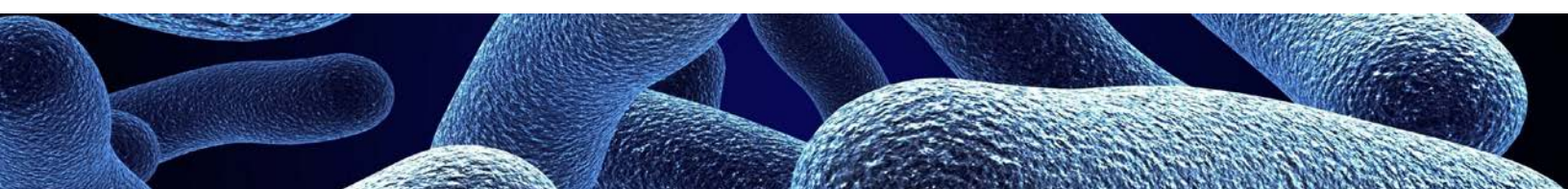

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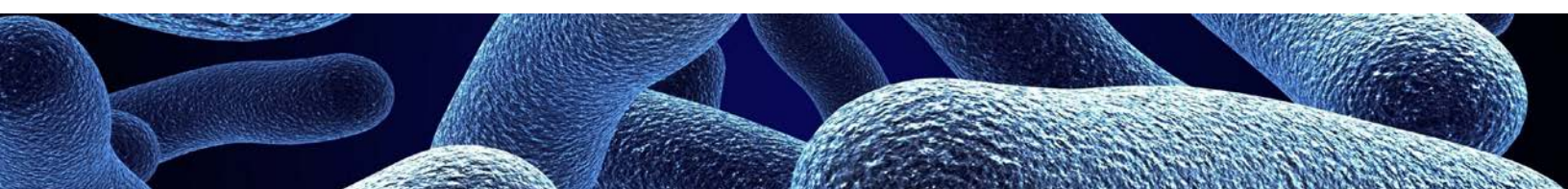

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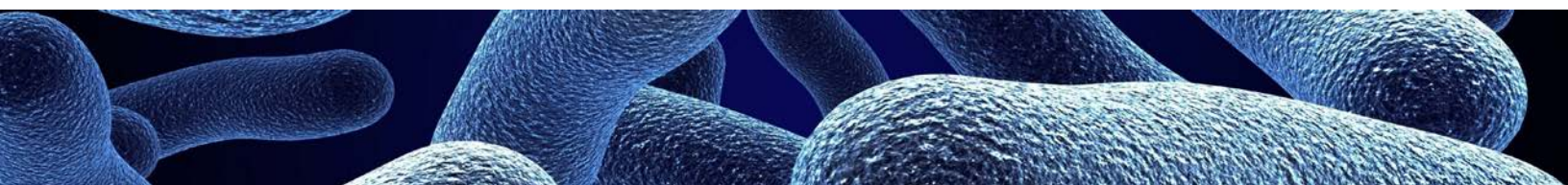

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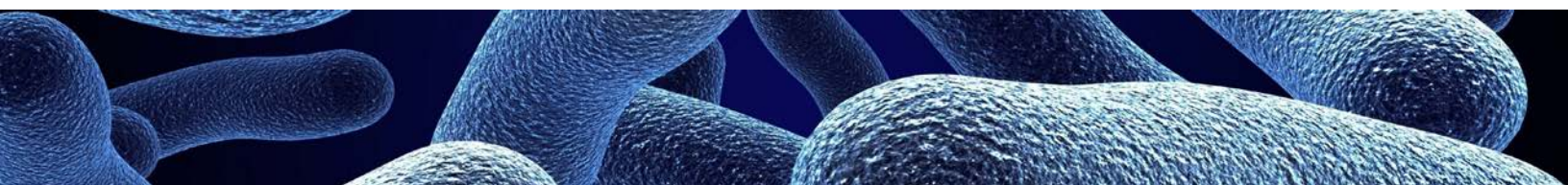

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