STANDING AT THE DAWN OF A POST-ANTIBIOTIC ERA

Simon Crox[1]

[1] Master Student Medicine, Radboud university medical center, Nijmegen, the Netherlands.

Perspective

Notes from the field hospitals at the time of World War I (1914-1918) reveal the horrors of an era without antibiotics. The discovery of penicillin by Alexander Fleming would happen a decade later. "The surgeon's one aim was to open up and clean out the wound, or to cut off the mortifying limb before the dread gangrene had tracked its way into the vital parts of the body" (Crofton E.1997, *p. 270-271*) [1]. Of course, this was also due to the high number of severe casualties and wounds contaminated with soil and shrapnel. Although systemic treatment was not an option, doctors were still very resourceful, and wounds were treated with local antiseptics. "I remember one particular case that had multiple injuries... it involved the genital organs and the anus. That was a very bad case and of course, it turned septic like everything else. We had to, in the end, put him in a bath, slung in a bath of potassium permanganate and kept him in that all the time. Just lifted him out to clean it up and put him back in again. The wound was permanently underneath, immersed in this solution" (Major General Escritt,1979, *letter, the Liddle collection*) [1]. The modern era of antibiotics has transformed medicine and saved millions of lives. However, the effectiveness of antibiotics is being endangered by the rapid emergence and spreading of resistant bacteria.

Introduction

In 1928 Alexander Fleming looked at a Petri dish, contaminated with mould, to see that it killed the bacteria he was examining. This event started the development of penicillin, which would be first prescribed to treat infections around 1940. A recent report from the United States Centers for Disease Control and Prevention illustrates that resistance is not a recent problem [2]. Within the year of the introduction of penicillin, a resistant strain of *Staphylococcus* was already identified. The occurrence of resistance stimulated the pharmaceutical industry to introduce new antibiotics [3]. Unfortunately, bacteria eventually seem to be able to form resistance to nearly all antibiotics that have been developed [3]. Within two years after introduction of methicillin, a methicillin-resistant *Staphylococcus aureus* (MRSA) was identified in 1962 [2]. Vancomycin, a second-line antibiotic, seemed untouched for almost two decades until resistance was documented in 1988 (Vancomycin-resistant *Enterococcus*; VRE) [2]. Around the same time, bacteria started producing extended-spectrum β-lactamases (ESBL), which makes them resistant to most penicillins and cephalosporins [2, 3]. The latter class of antibiotics is an important class for treatment of severe infections in hospitals. Shortly after, resistance was seen to carbapenems (Carbapenemase-producing *Enterobacteriaceae*; CPE), considered a last resort antibiotic, making CPE resistant to nearly all antibiotics [2, 3]. This article will further address the magnitude of the problem today, describing the importance of surveillance and give an overview of what could be expected in the future.

A Global Problem

There are several factors contributing to the emergence of resistant microorganisms. There is a clear relationship between overuse of antibiotics and dissemination of resistant strains [3]. Antibiotics decimate the susceptible microorganisms in a microbiome (i.e. the gut or skin flora) leaving the resistant strains to reproduce. Genes encoding the bacteria's resistance can also be transferred horizontally among both different and related species, using a 'mobile genetic' carriage system called plasmid. Prescribing the wrong type of antibiotic further promotes resistance [3]. Also, incorrect dosage or duration could promote gene alteration, due to subtherapeutic concentrations.

The World Health Organisation (WHO) identified all the actors at play and calls for worldwide action [4]. Every individual can help reduce infection load by following doctor's prescriptions closely (i.e. no 'over-the-counter' use), preventing infection through hygiene measures (especially healthcare workers) and keeping up-to-date with vaccinations. Policymakers should play their part in creating national plans, including financing systems for investment in new antibiotics, vaccines and diagnostic tools, and contribute to international programmes to tackle antibiotic resistance. Finally, the agriculture sector should cut back on its extensive antibiotic use in the veterinary sector and the spraying of crops. An astonishing 80% of antibiotics sold in the United States (U.S.) are being used in agriculture as it yields higher production [3]. The Netherlands already cut back extensively by reducing its use with 63% since 2009. Moreover, antibiotic residues, found in soil, wastewater and manure, originating from its use in agriculture, also exert a selective pressure in favour of resistant strains in nature's microbiome [5]. In the end, these resistant bacteria can find their way to humans. Access to preventive measures (proper sanitation, vaccines and disinfectants) and diagnostics tool for targeted treatment is more scarce in lower income countries. Overviewing the requirements to combat this crisis, it could well be argued that antibiotic resistance is also a problem of poverty [6].

To illustrate the need for global action, reports are published to raise awareness. It is estimated that 700,000 people worldwide die annually from resistant strains of bacteria, human immunodeficiency virus, tuberculosis and malaria put together [6]. For the U.S. and Europe the combined estimated annual number of deaths due to resistant bacteria is around 50,000 (e.g. a fully booked Johan Cruijff ArenA) [2, 6]. A meta-analysis of English literature between 1980 and 2000 found that the risk of death due to MRSA is 40% higher compared to its non-resistant form [7]. An increasing number of countries adhere to evidence-based guidelines annually. But, there is still a lot of room for improvement as only 19 countries (including the Netherlands) are currently at level five (the highest level) of their national antimicrobial action plan, called for by the WHO [8].

The A-team

The situation in the Netherlands is not as dire as it is assumed from these international numbers. Still, the Netherlands is not untouched, but we are mainly dealing with longer hospitalisation. This is explained by the need for relative toxic antibiotics, some of which can only be administered intravenously, in patients with infections with resistant bacteria [9]. CPE is rarely seen in the Netherlands, but in 2015 there was an outbreak of

29 CPE positive patients of unknown origin in a Dutch teaching hospital [10]. Hospitalisation abroad is the biggest risk factor for CPE carriage, emphasising the need for surveillance. Also, an astonishing 75% of Dutch travellers will be colonised with ESBL, for a median length of 30 days, after visiting India, revealing the price we pay for the current level of globalisation [11]. Therefore, a big portion of antibiotic resistance prevention in the Netherlands is focused on 'keeping the bad bugs out'.

Our own antimicrobial stewardship is covered by the so-called 'A-teams' operating within all Dutch hospitals, since 2014 [9]. They play a key role through pro-active surveillance of antibiotics use. In the Radboudumc, the A-team monitors the use of antibiotics, promotes the switch from intravenous to oral submission and warrants therapeutic drug levels [9]. Current evidence shows that this kind of antimicrobial stewardship decreases the length of hospitalisation [12].

New Therapies

New antibiotics are needed to tackle the current crisis. However, less than five percent of pharmaceutical investment is used for antimicrobial development [6]. It seems unprofitable to invest in a market when there is a worldwide call to cut back on its product. Therefore, it is important to create a new system in which developers are properly rewarded for their product [6]. On the other hand, we can look at alternatives to antibiotics. A recent review in The Lancet identified 19 'candidates', meaning they mostly still require basic research and translation in clinical trials, in various degrees [13]. We will now further look into a few promising alternatives.

Antibodies are widely used in medicine, because of their specific binding properties. This exploitation of the natural human immune system goes for treating autoimmune diseases, cancer and skin disorders among others, but could also further aid in treating infectious diseases when administered passively. For example, when bowel bacteria are decimated by antibiotics, *Clostridium difficile* sometimes grows in pathogenic proportions. Bezlotoxumab (a registered engineered antibody) binds and neutralises the toxins produced by *Clostridium difficile*, providing temporary passive immunity against infection

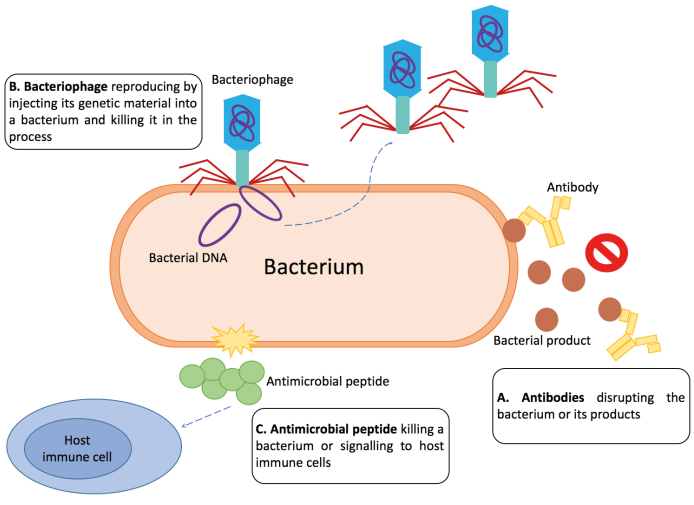

Figure 1: An overview of possible alternatives to antibiotics.

A: *Antibodies can be used to disrupt the bacterium in its environment (e.g. communication) or by binding to its products (e.g. toxins), amongst others.* ***B:*** *A bacteriophage infects a bacterium by injecting its own genetic material. The reproduction of new bacteriophages will eventually kill the bacterium as they burst out to find new bacteria to infect.* ***C:*** *These complex peptides, called antimicrobial peptides, naturally occur in our bodies and those of other multi-cell organisms. They can both modulate immune response (e.g. chemotaxis) and have bactericidal properties, meaning they can kill the bacterium. Unlike bacteriophages, they have very low specificity.*

caused by these toxins [14]. Another strategy, proven beneficial in a mouse model, is the disruption of communication between bacteria using antibodies causing less severe infection (figure 1A) [15].

Bacteriophages are another popular mentioned alternative to antibiotics. These so-called bacteria infecting-viruses occur throughout nature. In contrast to antibiotics, they tend to be very specific for the species as well as the strain of bacteria (figure 1B). Thereby, it is a potentially useful strategy if the bacterium causing an infection is identified, but imposes a problem if not, because then one would require a cocktail of bacteriophages [16]. Other challenges like rapid elimination by the body or development of resistance could be overcome by genetically engineering bacteriophages [13]. More research is needed to better understand the interaction between bacteriophage, human host (pharmacokinetics) and microbiome before large-scale implementation [16]. The earliest anticipated registration for clinical use is around 2023 in the U.S. and Western Europe [13]. Another take on fighting bacteria in this area of research is using just the purified lysins, which are the bactericidal enzymes produced by bacteriophages [13, 16]. *For more information take a look at our news item on the 4th of February 2018 on www.ramsresearch.nl*

Another promising area of research is the use of antimicrobial peptides. These peptides are naturally occurring in numerous multi-cell organisms aiding in host defence by binding to the plasma membrane of susceptible microbes. Apart from bactericidal properties, they are also able to modulate host immune response (figure 1C). Moreover, they are effective at very low concentrations and have a very broad spectrum of activity. Alike other alternatives, this group imposes many obstacles if used systemically, namely, proteolysis in the digestive tract, toxicity against red blood cells (and other host membranes) and high costs [17].

Most likely, alternatives to antibiotics will not serve as monotherapy right away, but rather as adjuvants in their first clinical trials, and prove their efficacy. Some alternatives could aid in preventing bacterial infections themselves, like vaccines. In the long run, combinations of alternatives or even monotherapy could be effective to combat pathogenic bacteria [13]. The need for new antibiotics and alternatives in the Netherlands right now is not pressingly urgent (in contrast to other parts in the world), but with a sustained rising number of resistant bacteria, we will eventually need them in the future [18]. Until then, it is important to preserve our current antibiotic effectiveness as much as possible for many years to come.

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Conclusions

With the emergence of CPE, which is resistant to almost all antibiotics, one could argue that we are standing at the dawn of a post-antibiotic era. In the Netherlands, the current antibiotics have still got a lot of life in them, by means of careful prescription and good surveillance. Still, with modern levels of globalisation this is a global problem and it is top priority for the WHO. New antibiotics are needed and, therefore, a new investment system is needed. Also, extensive research has provided a few alternatives to traditional antibiotics and some are expected to be used in clinics within a decade. Most promising alternatives include antibody therapy, bacteriophages and antimicrobial peptides, but most are still in a pre-clinical phase, making current antibiotics still the backbone of combating bacteria.

References

1. Runcie, H. Infection in a Pre-Antibiotic Era. Journal of Ancient Diseases & Preventive Remedies 03(2015).
2. Centers for Disease Control and Prevention, Office of Infectious Disease. Antibiotic resistance threats in the United States, 2013. Retrieved from: http://www.cdc.gov/drugresistance/threat-report-2013 (Accessed: 21st October 2018).
3. Ventola, C.L. The antibiotic resistance crisis: part 1: causes and threats. P T 40, 277-283 (2015).
4. World Health Organization. Fact sheets on antimicrobial resistance (2018). Retrieved from: http://www.who.int/news-room/fact-sheets/detail/antibiotic-resistance (Accessed: 23rd October 2018).
5. M.G. Pikkemaat, H.Yassin, H.J. Van Der Fels-Klerx, B.J.A. Berendsen. Antibiotic Residues and Resistance in the Environment. RIKILT report (2016).
6. O'Neill J. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. Rev Antimicrob Resist. 2014. Retrieved from: http://amr-review.org/Publications (Accessed: 25th November 2018).
7. Cosgrove, S.E., et al. Comparison of mortality associated with methicillin-resistant and methicillin-susceptible Staphylococcus aureus bacteremia: a meta-analysis. Clin Infect Dis 36, 53-59 (2003).
8. World Health Organization. Monitoring Global Progress On Addressing Antimicrobial Resistance (AMR): Analysis report of the second round of results of AMR country self-assessment survey. WHO report (2018).
9. Hulscher, M., et al. Microben bestrijden doe je zo (2017). Retrieved from: https://www.medischcontact.nl/nieuws/laatste-nieuws/artikel/microben-bestrijden-doe-je-zo.htm (Accessed: 26th October 2018).
10. Leenstra, T.E.A., et al Carbapenemase-producerende enterobacteriën in Nederland. Ned Tijdschr Geneeskd 161(2017).
11. Arcilla, M.S., et al. Import and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. Lancet Infect Dis 17, 78-85 (2017).
12. Schuts EC, et al. Current evidence on hospital antimicrobial stewardship objectives: a systematic review and meta-analysis. Lancet Infect Dis. 16(7): 847-56 (2016).
13. Czaplewski, L., et al. Alternatives to antibiotics—a pipeline portfolio review. The Lancet Infectious Diseases 16, 239-251 (2016).
14. Farmacotherapeutisch Kompas, Bezlotoxumab (2018). Retrieved from: https://www.farmacotherapeutischkompas.nl/bladeren/preparaatteksten/b/bezlotoxumab (Accessed: 18th November 2018).
15. Palliyil, S., et al. High-sensitivity monoclonal antibodies specific for homoserine lactones protect mice from lethal Pseudomonas aeruginosa infections. Appl Environ Microbiol 80, 462-469 (2014).
16. Van der Meer, J.W.M. et al. Bacteriofagen in de strijd tegen multiresistente bacteriën. Ned Tijdschr Geneeskd 162(2018).
17. Walkenhorst, W.F. Using adjuvants and environmental factors to modulate the activity of antimicrobial peptides. Biochim Biophys Acta 1858, 926-935 (2016).
18. De Greeff, S. & Mouton, J. NethMap 2018: Consumption of antimicrobial agents and antimicrobial resistance among medically important bacteria in the Netherlands / MARAN 2018: Monitoring of Antimicrobial Resistance and Antibiotic Usage in Animals in the Netherlands in 2017. RIVM Rapport (2018).

CORRECT ANSWERS TO THE EXAM QUESTIONS

Answer question 1:
A. Efflux

The efflux mechanism entails resistance towards tetracyclines, which is often plasmid-mediated and attributable either to active efflux pumps. These are generally specific for tetracyclines or proteins that protect the ribosome from tetracycline action. With this mechanism, tetracyclines are removed from the bacterial cell by increased expression of efflux pumps. Enzymatic changes as a resistance mechanism can occur in antibiotics such as fosfomycin and aminoglycosides. Higher production of target binding sites as a resistance mechanism occurs in glycopeptides and lipoglycopeptides, while lower membrane permeability occurs in β-lactams.

For further reading: Hooper, D.C. Bacterial Resistance to Antimicrobial Agents in Harrison's Principles of Internal Medicine, 20e, Vol. (McGraw-Hill Education, New York, NY, 2018)

During the exam, 35% of the participants answered this question correctly.

Answer question 2:
B. Filters encapsulated bacteria from the blood circulation

In the absence of a spleen (asplenia), the natural filtration of microbes in the blood does not take place and leads to a predisposition to fulminant infections by encapsulated bacteria. Asplenia leads to lower production of antibodies since the proliferation and differentiation of antibody-producing plasma cells take place in the spleen and lymph nodes. Complement factors are specifically synthesised by the liver, not the spleen. Also, the complement system is responsible for opsonisation by phagocytosing cells, not lysis. Splenic macrophages and dendritic cells are responsible for the uptake of microorganisms and microbial products in the blood. The macrophages and dendritic cells then stimulate the B and T cells that arrive in the spleen from the blood. Natural killer cells can kill encapsulated bacteria when these are opsonised with antibodies (antibody-dependent cell-mediated cytotoxicity), but this is not specific to the spleen.

For further reading: Fischer, A. Primary Immune Deficiency Diseases in Harrison's Principles of Internal Medicine, 20e, Vol. (McGraw-Hill Education, New York, NY, 2018) and Parham, P. Elements of the Immune System and their Roles in Defense in The Immune System, 4e (Garland Science, New York, NY, 2014)

During the exam, 48% of the participants answered this question correctly.

The exam questions can be found back on page 7 in this journal.