Why is the development of new antibiotics so difficult?

Life without antibiotics would be unimaginable. Considering the stark increase in population over the past century, human existence would have been cut incredibly short if it were not for these powerful, cleverly designed biochemicals. Human life expectancy has increased by a remarkable 23 years since the use of antibiotics![1] But with rising discoveries of antibiotics during the Golden Age, additional to their uncontrolled overuse in clinical situations, the ominous probability of antibiotic resistance cropping up was undeniable. Unfortunately, to this day, finding potential new antibiotics is a much larger task than it was during the Golden Age of antibiotics, but is necessary if we are to eradicate the most notorious and resistant bacterial pathogenic diseases on this planet.

The development of antibiotics in the current generation is by far one of the ultimate challenges that scientists face. With the rapid surge in variants of multiple diseases across the globe killing 700,000 people every year, the antibiotics we have alone are far from capable in combatting these rising threats.[2] Antibiotic resistance of many bacterial strains is mainly to blame for the difficulty in forming new treatments, additional to the immense economic factor and timescale of events that come along with developing antibiotics. If antibiotic resistance pursues at its current rate, it will greatly contribute to the predicted 10 million deaths expected by 2050 from antimicrobial resistance.[3]

The origin of antimicrobial usage surprisingly dates back thousands of years. Around 2000 years ago, mouldy bread was used to treat open wounds among ancient civilisations in countries including China and Egypt.[1] The oldest medical document preserved about traditional remedies was that of Eber's Papyrus from 1550 BC, where medicinal soil and bread were used as microbe treatments.[1] Natural resources used as ailments was practice long before the extraction of many antibiotic products.

Sir Alexander Fleming's astounding discovery of the penicillin antibiotic in 1928 from observing colonies that had formed of previously prepared petri dishes was a ground- breaking revolution in terms of antimicrobial knowledge, as it had a major role in treating many bacterial penicillinases.[2] Its discovery foreshadowed the Golden Age of antibiotics between the 1940s and 1960s, where many natural products were discovered and clinically used. The additional understanding of penicillin's structure is heavily credited to Dorothy Hodgkin, whose use of x-ray crystallography in 1945 confirmed its β-lactam structure.[1]

Antibiotics previously discovered which were and still are in use in the pharmaceutical industry mainly arose from natural products of microbial species. There is immense chemical diversity between natural product antibiotics. A particular group of bacteria, of which are responsible for 64% of natural product classes discovered and used, are known as filamentous actinomycetes.[1] The early discovery of the aminoglycosides class in 1944 targets the 30S subunit of bacterial ribosomes during protein synthesis. Other bacterial species can produce natural products including polypeptides, such as Gramicidin A from the bacterial species *Bacillus brevis*.[1] One of the infamous classes of antibiotics, penicillins, originates as a natural product of fungal species, including *Penicillium chrysogenum,* from which the semi- synthetic drug amoxicillin was produced in 1929. Its main function is disrupting cell wall synthesis in bacteria, by affecting penicillin- binding proteins.[1] However, the misuse of discovered antibiotics clinically hinders

their lifetime of effectiveness. This is seen with the aminoglycosides, to which targeted bacteria resist via processes including phosphorylation and acetylation.[4] As a result of increased antibiotic resistance due to the Golden Age, many synthetic antibiotics have been formed, including sulphonamides. Discovered by Gerhard Domagk, sulphonamides were thought of as the first proper antimicrobials that could be used against a range of bacterial pathogens.[5] Synthetic antibiotics are heavily based on the chemical structure of natural products and have been the only sorts of antibiotics produced since 1984.[2]

Antibiotics make many surgical procedures within hospitals possible. Surviving caesarean sections, organ transplants and cancer surgeries, for example, would be extremely unlikely if it were not for the use of antibiotics in nosocomial environments. Despite the immense array of natural and synthetic antibiotics available today, many of the mechanisms of these compounds are being overwhelmed by the dreaded antibiotic resistance. Mutant strains of pathogenic organisms have risen in hospitals, leading to deadly variations of diseases being spread amongst patients and further communities, as observed with *Mycobacterium tuberculosis*.[4] Transferable resistance against antibiotics had been speculated for a long time, and one of the reasons for vast dispersions of antibiotic resistance amongst bacteria is due to horizontal gene transfer (HGT). The idea is corroborated by the prominence of bacterial gene sequences found within the genomes of eukaryotic cells, suggesting that this mechanism is used to spread favourable genes amongst bacterial communities.[4] HGT is a mechanism by which bacterial species obtain useful genes interspecifically, such as passing from environmental species to pathogenic ones.[6] Gene transfer can occur via the transduction of bacteriophages, conjugation of plasmids or natural extracellular DNA transformation.[7] Many superbugs form at faster rates due to the propulsion of resistance via HGT, as opposed to spontaneous genetic mutations. Bacterial activities within hospital environments are highly governed by factors including daily usage of disinfectants and regular contact between healthcare staff and patients.[6] Infections in hospital environments from antibiotic resistant bacterial pathogens have a higher chance in being fatal, especially to those exposed with weaker immune systems.

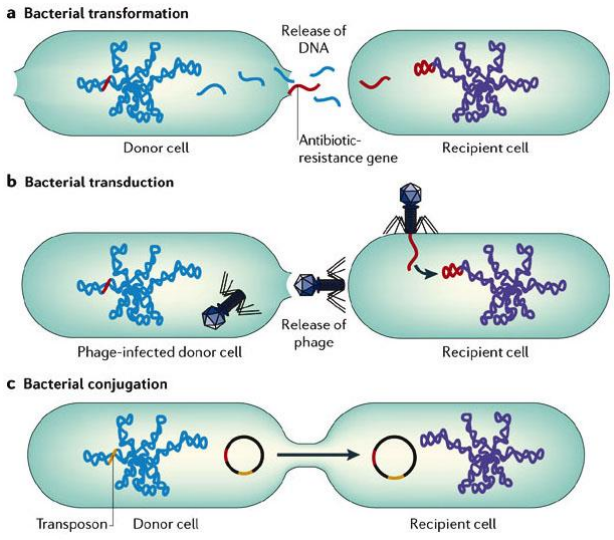

Fig. 1 Three mechanisms of HGT[9]

Plasmid mediated transfer via conjugation is the most prominent form of HGT, where two bacterial cells of the same environment must be in physical contact to transfer the plasmid from one cell to another. This is seen against β- lactams, where the resistance genes of lactamases are commonly found on plasmids.[7] Natural transformation- exhibited by around 1% of bacterial species- results in the antibiotic resistant genes being combined chromosomally with the bacterial cell's genome, making these harder to detect.[8] Transduction involves the transfer of bacterial genes integrated with recombinant viral genomes of bacteriophages, as part of the lysogenic cycle.[9] Studies have suggested that over 70% of bacterial faecal samples contain traces of antibiotic resistant genes.[7] HGT increases the resistance of many pathogenic species in clinical environments, making them more persistent in nosocomial situations, and greatly

reducing the effect of currently used antibiotics. As a result, the development of antibiotics for clinical environments is much more difficult than thought, due to the dramatic rise in HGT and dispersion of antibiotic resistant strains across the community.

Superbugs continue to plough unstoppably along their paths to invincibility due to the excessive use of antibiotics amongst humanity. They have an unbounded resistance to an immense variety of existing antibiotics that are meant to target them, thus are more virulent and highly transmissible.[10] Many bacterial strains avert multiple drugs (multi-drug resistant strains). Some species, including *Mycobacterium tuberculosis* shows strains of extremely drug resistant (XDR) character, undoubtedly due to their rapidly changing genome which has evolved for years alongside humans. The clustering of resistance genes in some organisms due to various HGT mechanisms, along with frequent unexpected mutations, have provoked a build-up of very resistant strains.[7]

MDR strains are detrimental to patients who are more susceptible to obtaining nosocomial diseases. This is seen with the indisputable risk that many Cystic Fibrosis (CF) patients have against the Gram- negative pathogen *Pseudomonas aeruginosa*. Due to the higher vulnerability of CF patients' immune systems, they are usually assigned to a lengthy course of using antibiotics, which is a stimulative source for antibiotic resistance.[4] Developing antibiotics against MDR and XDR strains is an immensely taxing ask. It is harder to develop effective biochemicals, considering how better adapted previously targeted sites on bacteria are to antibiotics. The job is made incredibly difficult due to the rapid spread of these strains amongst healthcare environments and to the wider community where tracking becomes challenging.

As important as nosocomial areas are in the spread of antibiotic resistant genes, it is vital to consider situations accounting for this distribution globally. The overpopulation of superbugs can be widely linked to cases of civil unrest, famine, natural disaster, and poor practice within healthcare facilities.[4] These have resulted in incredibly high mortality rates and an uncontrollable spread of some diseases across the world. Whilst industrialised countries have sophisticated technologies, more hygienic clinical environments and better- tested treatments, many low-income countries have a higher number of fatalities due to an uneven distribution of these resources internationally. This is observed with the water transmissible bacterial pathogen *Vibrio cholerae*, causative of cholera. There is an observable stark difference in cases between the UK (which has safe water and regulated sewage systems) and less developed countries within Asia.[4] To deal with this, more than 70 million oral cholera vaccines have been administered from mass vaccination campaigns to areas of severe outbreak, including those under humanitarian crisis.[11] A substantial problem with developing effective antibiotic treatments for globally spread MDRs and XDRs is the lack in tracking the distribution and levels of serious infectious outbreaks. In remote areas, there is infinitesimal amounts of

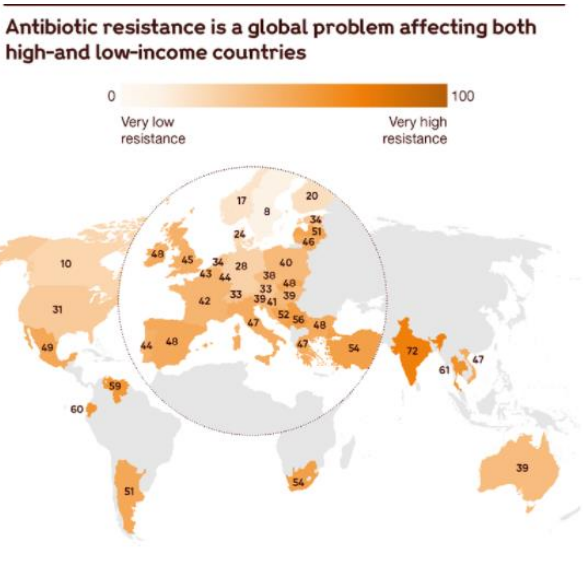

Fig. 2 Variance in antibiotic resistance globally[16]

reporting, meaning less detection and awareness of outbreaks there. Following this, it is harder for major medical organisations, including the World Health Organisation to identify any new, virulent, and persistent strains from other countries, for which new antibiotics must be developed.[4,6]

Anthropogenic activities have had a staggering impact on the level of antibiotic resistance observed today. Human actions have jeopardised how effective antibiotics can be, due to their exploitation in many non- therapeutic scenarios. Their using for animals, pest control within agriculture and plant cloning are just some of the examples of antibiotic usage away from medicinal purposes.[4] A shocking 73% of all antibiotics are used in the agricultural industry, which is bizarre considering their original purpose as a remedial source![12] The Earth's biosphere is continuously being polluted with antibiotics that are not correctly disposed of (including their appearance in untreated water effluents alongside organic wastes).[13] As a result of this overexposure, bacterial species are under higher selection pressures in the environment, so organisms with alleles for higher resistance are likely to survive and spread, making antibiotic development much harder.[6]

Synthesising new antibiotics or obtaining natural products is a strenuous task, occurring over an insane timescale. Finding a chemical strong enough to destroy pathogenic bacteria is by far simple- many chemicals are beyond capable enough of killing many of these microorganisms.[2] However, clinically approved antibiotics are used within the delicate, complex human body hence finding and testing chemicals can take 10 to 20 years before approval of their use.[2] The potential candidates must not be toxic to the body's internal environment, offering limited side effects from taking the course of antibiotics. As a result, the levels of toxicity of substances and their effect on normal biological pathways must be accounted for when researching into new antibiotics.

The continuous overriding of antibiotics due to natural evolution of bacteria makes these antimicrobials majorly off- putting to research. Instead, focus is being placed on the exploration of vaccination as protection against very harmful microbes.[2] Rather than investing money into antibiotic synthesis consistently, vaccination appears to be the anticipated route in treating future bacterial infections. Evaluations of antibiotics in development show that many of the 40 to 50 contenders do not provide significant benefits over pre-existing sources already in use.[14] It thus appears favoured that resources go towards more effective long- term immune treatments as opposed to developing more antibiotics that would only be outweighed by the inevitable adaptation of bacteria.

Investing into antibiotic markets are a huge risk. Research and development of effective antibiotics and the clinical trials of the potential antimicrobial candidates results in a very time-consuming, expensive process.[14] It costs approximately $1.5 billion dollars to get a drug into the market![15] However, the market for antibiotics nowadays is greatly stumped due to the inexorable evolution of bacterial pathogens, with fewer than 1 in 70 possible drugs being successful.[16] Many newly synthesised antibiotics must be used sparingly in the population to limit the prospective resistance developed against them. Antibiotics are generally sold at lower prices compared to other treatments.[16] Many large pharmaceutical companies have stopped investing in antibiotic production due to this because there is a substantial economic loss in the process. Most cannot make profits from creating these drugs, seen with the fall of biotechnology

companies such as Achaogen.[14] Instead, the vast majority (around 90%) of antibiotic development works are focused on in smaller companies, including CARB-X.[14]

Testing for many bacterial infections in nosocomial environments is neither quick nor sensitive. For instance, MIC testing to identify particular pathogens from using small concentrations of antibiotics, is hard to undergo if the location of the infection is hard to access. Additionally, cultures of microbes need to be grown from the taken sample, which can take a long time.[17] As a result, many doctors prescribe broad spectrum antibiotics to patients, which may not necessarily be useful to the patients' infections. It may be that the patient does not have a serious infection requiring a large antibiotic course, or antibiotics at all.[18] According to Public Health England, 20% of antibiotic prescriptions are unnecessary.[2] It could also be proposed that the patient is suffering from a far more resistant bacterial infection, requiring a more specific form of treatment that the antibiotic given. Either way, larger exposure of the patients' internal environments to more antibiotics can cause rise of more resistant bacterial strains.

Evidently, there are several premises making antibiotic development in the future greatly difficult, many primarily stemming from antibiotic resistance. More insight into the dynamic routes leading to resistant genomes amongst these pathogens is required, including research into HGT by using genetic markers to identify DNA mobility amongst bacterial organisms.[4] This could provide a source from which more specific antibiotics can be designed, with longer lasting effects in the body. Additionally, alternative methods are being taken to reducing antibiotic resistance in the near future, such as vaccine rollouts, reduced antibiotic prescription and better dealing with disposal of antibiotics that would otherwise end up contaminating the environment. This is already seen within clinical commissioning groups trying to reduce prescribed antibiotics in primary care situations by 4%.[19] Increased public awareness on sanitation is vital in reducing the spread of microbial infections.[2] More funding should be put into developing faster, distinctive diagnoses.[14] Furthermore, support is needed from the government, who must acknowledge the serious consequences of resistance, hence should work alongside philanthropic organisations (such as TB Alliance, who created Pretomanid for XDR TB strains[20]) to help bring more drugs or alternative solutions to the current declining market.[14]

Ultimately, antibiotic shortages must be acknowledged as a worldwide problem. If not dealt with methodically or efficiently, the consequences of undefeatable pathogens could face us and future generations to come. Antibiotic production is reasonably dwindling, but with research into bacterial gene transfer and increasing awareness of resistance, new prospective treatments away from antibiotic development are in sight. However, we must all collectively and collaboratively act now, reducing our reliance on antibiotics, to savour them for as long as we can.

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