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The Whys and Wherefores of Antibiotic Resistance

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The development and rapid dissemination of antibiotic-resistant bacterial pathogens has tarnished the dream of a world without infectious diseases. However, our understanding of these processes, paired with sequence information from terrestrial bacterial populations, indicates that there is no shortage of novel natural products that could be developed into new medicines. Regardless, their therapeutic success in the clinic will depend on the introduction of mandatory controls and use restrictions.

One solution to control the threat of antimicrobial resistance is scientific discovery.

—Dame Sally Davies

The history of man has been punctuated by many plagues and pestilences during existence on this planet (see Table 1 for a partial list). In certain instances, upward of 50% of the population of a city or a country may have perished while others survived, albeit seriously weakened. Plagues have shaped history at both local and worldwide levels from an economic point of view by crippling the workforce, and also from a military viewpoint, infections on one side in a conflict could lead to victory over the enemy. In recent times, the most devastating microbial infection was the Spanish flu pandemic in 1918–1920 that killed some 5% of the world's population. However, in the past 50 years, the world has seen the rapid evolution of a new plague—that of worldwide antibiotic-resistant (AR) microbes. Although not a disease in itself, AR results from the failure to effectively prevent and treat many diseases, leading to widespread untreatable microbial infections and greatly increased morbidity and mortality: a plague of resistance genes (Davies and Davies 2010). AR transforms treatable infectious diseases into untreatable ones. Regrettably, we were warned and aware of the cause and consequences of AR development and dissemination in the 1960s, but nonetheless we let it happen!

Bacterial pathogens readily develop resistance on treatment with antibiotics; they are often caused by the results of mutation of specific target genes but primarily by the inheritance of plasmids carrying resistance gene clusters (Wright 2011). Multidrug-resistant pathogens have been clinically relevant since the 1950s and can be considered the leading cause of mortality worldwide since the introduction of antibiotics (Davies and Smith 1978). Currently, estimates indicate that upward of 10 million

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Table 1. History of plagues

Death toll (estimate)	Location	Date	Comment	Disease
ca. 40% of population	Europe	541–542	Plague of Justinian, attributable to the name of the Byzantine emperor in power at the time	Bubonic plague
30% to 70% of population	Europe	1346–1350	"Black Death" or second plague pandemic, first return of the plague to Europe after the Justinianic plague of the 6th century	Plague
100,000	England	1665–1666	Great plague of London	Plague
76,000	Austria	1679	Great plague of Vienna	Plague
>50,000	Russia	1770–1772	Russian plague of 1770–1772	Plague
>>100,000	Asia, Europe	1816–1826	First cholera pandemic	Cholera
>>100,000	Asia, Europe, North America	1829–1851	Second cholera pandemic	Cholera
20,000+	Canada	1847–1848	Typhus epidemic of 1847	Epidemic typhus
1,000,000	Russia	1852–1860	Third cholera pandemic	Cholera
1,000,000	Worldwide	1889–1890	1889–1890 flu pandemic	Influenza
75,000,000	Worldwide	1918–1920	1918 flu pandemic	Influenza
2,000,000	Worldwide	1957–1958	Asian flu	Influenza
1,000,000	Worldwide	1968–1969	Hong Kong flu	Influenza
775	Asia	2002–2003	SARS	SARS coronavirus
14,286	Worldwide	2009	2009 flu pandemic	Influenza
Incalculable	Worldwide	1950-	Antibiotic resistance	Infectious diseases

deaths occur every year attributable to AR, and this number is increasing. The financial costs associated with treating these intractable infections are in the many billions of dollars, and the emotional cost has been enormous (Davies et al. 2014). Indeed, many infected patients in the population may well be treated successfully, but antibiotic overuse and misuse propagates resistance development and further mortality from worsening, untreatable conditions. We have been using antibiotics in ever-increasing amounts for the last half century, and it is estimated that the AR plague may have claimed >500 million lives worldwide.

The AR plague has broad sequelae: Domesticated animals such as pets and farm animals also die of infection with AR pathogens. This contributes to the global AR gene pool (Bush et al. 2011). Furthermore, we have little or no notion as to the effects of antibiotics and resistance on the global microbial ecosystem that provides endless services to humans (Colwell 1997). The worldwide economic consequences of AR are difficult to evaluate; meanwhile, antibiotics have not eliminated a single microbial disease.

A BRIEF HISTORICAL PERSPECTIVE

In all probability, microbial resistance to toxic molecules occurred long before the modern era of antibiotics. Indeed, agents such as arsenicals and mercurials were used for centuries in treating disease even while the role of microbes in causing these afflictions was not recognized. Often, treatment failure was more likely caused by the toxicity of the potion rather than the disease. It is not coincidental that many antibiotic resistance plasmids isolated in the 1950s carried genes for resistance to salts of arsenic and mercury (Silver and Misra 1988). There is also a real possibility that genetically determined resistance was spread during the intense pollution in the late 18th and early 19th centuries, when the environmental microbiomes of the industrial world were exposed to huge amounts of

toxic organic chemicals. Not everyone lived in Downton Abbey! Respiratory diseases were very common in industrial areas like Manchester, Pittsburgh, and in other countries throughout the world. Currently, many cities in Asia suffer the same problem. What immediate and lasting impacts might this pollution have on human microbiomes?

SOME ANTIMICROBIAL HISTORY

The true antibiotic era began with the discovery of penicillin (a fungal product) by Fleming in the late 1920s, but serious studies did not commence until the 1930s (Abraham and Chain 1988). By the 1930s, most of the common infectious diseases were identified as microbial in origin and specific treatments could be applied. The first significant treatment discovery was that of the sulfonamides, a class of synthetic antimicrobial agents introduced into clinical practice in 1935. Sulfonamides were inexpensive, easily produced in large amounts, and were widely used in different forms and, together with penicillin, played an incredibly important role for the Allied forces in the Second World War preventing many deaths. Winston Churchill was treated successfully for bacterial pneumonia with sulfapyridine; this was obviously of great importance! Sulfonamide resistance was undoubtedly encountered in certain cases but there are few descriptions in the early literature; the biochemical and genetic mechanism(s) involved were not studied at the time. The situation has changed and mechanisms of action and resistance to the sulfonamide drugs have been well characterized (Sköld 2000). For example, antibiotic resistance integrons encoding sulfonamide resistance were unrecognized until the isolation of multiply drug-resistant strains in postwar Japan (Davies 1995). Sulfonamides are still in use today although less frequently.

Trimethoprim is another synthetic agent that was developed in the early 1960s and, like the sulfa drugs, has enjoyed a long life. It is often used in combination with a sulfonamide for the treatment of urinary tract infections, and dual resistance was first identified in 1969. Within the context of this short article, it is worth noting that resistance to sulfa drugs and trimethoprim can occur by mutations in the target pathways or by inheritance of plasmids carrying altered, drug-insensitive variants of the target enzymes (Sköld 2001). Very few examples are required to see that AR mechanisms are extremely varied and widely distributed.

It is often forgotten that *Bacillus* strains were studied early on and found to produce some of the first bioactive molecules, of which a number have proven to be useful therapeutics. These included polymyxin (the universal topical treatment) and colistin, currently one of the few drugs available for certain multidrug-resistant *Pseudomonas* infections. These compounds have been used for more than 80 years, and plasmid-borne colistin resistance has recently been observed to be widespread in China (Liu et al. 2016) (next stop, Europe and the United States). Sadly, there appears to have been little effort devoted to the discovery and development of bioactive compounds from *Bacillus* sp. and related genera in recent times. For some reason, pharmaceutical companies do not favor peptide drugs. These and other "forgotten" bacterial genera should become more fashionable now that their genome sequences can be scanned for biosynthetic pathways in the search for novel classes of bioactive compounds. Another topic of interest concerns the evolution of antibiotic resistance in the case of *Mycobacterium tuberculosis* (Mtb). The sole source of resistance to anti-TB drugs is by mutation of the target genes and no transferable antibiotic resistance plasmids have been found to encode resistance in Mtb: This pathogen seems to take care of antimicrobials quite nicely without participating in sex (Musser 1995).

THE CHEMICAL DEVELOPMENT OF ANTIBIOTICS

Since the discovery of antibiotic resistance genes that modify (inactivate) antibiotics, there has been considerable effort to develop, by synthetic chemical methods, compounds that prevent, inhibit, or otherwise avoid antibiotic resistance (Davies 2014). With each new resistance mech-

anism, we begin a search for blocking agents to restore antibiotic activity and there have been some remarkable successes. This often consists of the removal or modification of functional sites of antibiotic "core" structures to avoid modification or inactivation by resistance enzymes. A good example of this approach is the chemical modification of aminoglycosides to prevent phosphorylation or acetylation of sugar hydroxyl groups or amino groups (Davies 2006). In the case of amikacin, a structural element from a less effective aminoglycoside was added synthetically to kanamycin and prevented specific enzymatic modifications. The discovery of compounds such as amikacin were landmarks, but unfortunately not all resistance modifications can be avoided in this way and "Achilles heels" remain on the modified drugs (Courvalin and Davies 1977). Another example is the chemical modification of β-lactam antibiotics to prevent hydrolytic cleavage of the β-lactam ring, which has been successful in the case of the modern β-lactam antimicrobials. But again, modified β-lactamase enzymes (>1000) have evolved in concert with the development of new semi-synthetic β-lactams, and the conflict continues (Bush and Jacoby 2010). The case of the quinolones is especially interesting. When the fluoroquinolones were introduced, it was claimed by some that no resistance modification other than mutation of this new class of completely synthetic antimicrobials would be possible. Little did they know—microbes responded to this challenge successfully by adapting another enzyme for the job, and transferable resistance to the fluoroquinolones is now common in Gram-negative pathogens (Strahilevitz et al. 2009). Resistance is inevitable.

RESISTOMES

One of the most remarkable environmental findings of recent years is that of antibiotic resistomes: conglomerations of putative resistance genes isolated from a variety of environmental sources (Forsberg et al. 2012). This descriptive term was coined by Wright and his colleagues and confirmed and extended by the characterization of resistomes from many different environments (D'Costa et al. 2006). They include soils, ancient caves, and, not surprisingly, gut microbiomes—(putative) antibiotic resistance genes are everywhere. The question is, do these reservoirs play any role in the determination of clinically significant antibiotic resistance? The putative AR genes from resistomes have been shown to be active by gene-expression studies, but this does not establish their natural function. Indeed, the presence of AR genes in bacteria has been shown to influence many other phenotypes. Is it possible that these antibiotic resistance genes have different properties in the wild? Is there any evidence for association with plasmids? To date, the presence of natural resistomes appears to have no causal relationship with the use of antibiotics.

GENE TRANSFER

Antibiotic resistance might have been less of a problem were it not for the fact that most AR genes are genetically mobile. Resistance transfer factors (R-plasmids) appeared on the environmental/clinical scene in the 1950s in Japan, the United States, and Europe: Where did they originate? The combined interaction of mutation and gene transfer must have been taking place (with respect to resistance development) since eternity. There seems to be endless diversity with rampant gene exchange occurring within related groups of microbes and plasmids that are but one of the transfer mechanisms (Polz et al. 2013). Bacterial gene transfer has become prominent because of the use of antibiotics, and equally powerful selections were likely operating during the period of the industrial revolution (toxic chemicals and poisons). There is still much to be learned about gene transfer mechanisms and origins of AR genes. What is clear is that microbes are able to recombine promiscuously and readily access local gene pools. It is interesting to note that some recent studies of plasmids with strictly environmental roles show that they can be rapidly assembled and disseminated (Xue et al. 2015). Even with the most modern sequence data and analyses of resistance islands, it is not possible to trace the AR genes back to their origins (Ashton et al. 2015). What

are natural functions and origins of AR genes? If the resistomes have their supposed function, does one find antibiotic-producing organisms and plasmids in plenitude in resistome environments? There are resistomes in the human gut, but do their resistance phenotypes all relate to antibiotics being ingested? If a person has never been treated with antibiotics such as tetracycline, streptomycin, or chloramphenicol, etc., where did the resistance genes come from?

THE BIG MISTAKE

The current situation, wherein transferable multidrug resistance exists universally, was an inevitable consequence of the negligent treatment practices used (and to some extent continued) when antimicrobials were first introduced in the 1940s. Their use as therapeutics was generally successful, but there were many senseless practices. For example, using antibiotic fermentation residues (and crude antibiotics) as feed supplements in the beef, chicken, and fish industries have proven to be a contributor to the AR plague. Efforts have been made to control these misuses, but they have only been partly successful in a few countries, and the consequences of the worldwide commercial (not-health-related) use of antibiotics are now irreversible. Antibiotic production residues should never have been used in agriculture and as food supplements for animals and fish. In retrospect, it seems that everything possible was done to ensure that antimicrobial use was encouraged in as many nonhuman health practices as possible. These actions guaranteed that effective therapeutic applications would be severely limited. Alexander Fleming predicted this outcome but it is unlikely that even he realized what would happen on a global scale.

WHAT SHOULD BE DONE

1. Strict (legal) control of antibiotic use must be exercised (from compound discovery to the commercial release by the manufacturer) and all remaining residues must be eliminated. Proper hospital practices must be enforced to prevent overuse and disposal of antimicrobials in their active forms. Could antibiotics be destroyed before disposal? Constant monitoring by modern methods is needed within and without. But, how do you punish hospital personnel for noncompliance?
2. Nonhuman use of antibiotics: Be they for prevention, therapy, or for growth promotion, should all be universally banned except for specific agents that are structurally and completely unrelated in mode-of-action to compounds used for humans. Infractions must be disciplined appropriately. Under no circumstances should antibiotic use be permitted other than for human therapy. These measures should have already been adopted internationally.
3. Use of any agent that leads to the development of antibiotic cross-resistant strains, such as triclosan, should be banned, no matter what the proposed use. More research to identify chemicals that cross-select for resistance to antibiotics is essential. The same is true for agents where multiple resistance genes are becoming frequently cotransferred.
4. Should antibiotic use in genetic engineering been permitted? Significant quantities of antibiotics (although very small compared with human and animal use) and AR genes have been used for gene cloning by academia and industry since the early 1970s. The impact of this "misuse" on the advancement of biological science has been enormous and has led, and will lead, to future advances in medicine and biotechnology. The hullabaloo over genetic engineering practices is misplaced, but the extent to which it depends on the use of antibiotic and resistance selection should be considered.

In conclusion, is there reason for optimism for the future of antibiotic therapy? Of course there is, but the only solution is to generate collections of truly novel antibiotics with a narrow spectrum of action that can be combined with synthetic inhibitors of AR function. There is no shortage of potential therapeutic agents in nature; there are many new antibiotics to be dis-

covered, and current methodology comes nowhere near exhausting searches of natural environments. Creative screening approaches that rely on natural properties such as signaling will lead to a renewable supply of novel compounds. The same can be anticipated with bioinformatic-heterologous expression approaches (Donia and Fischbach 2015). However, the compounds will have short useful lives unless there is strict control of their use. AR is an evolutionary response by microbes that has had drastic consequences for the human race. We need to study the origins of AR and elucidate their "natural" functions. A key component will be to understand how AR diversity is generated as a result of rapid gene transfer and turnover. Finally, it must be recognized that the AR plague was entirely man-made and could/should have been prevented and/or contained by stricter control of the use of antibiotics. Without appropriate compliance of regulations, it is unlikely that the spread of AR will ever be prevented.

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