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The ways in which bacteria resist antibiotics

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Abstract. The array of mechanisms that bacteria possess to withstand extreme conditions and to resist harmful drugs and other toxic agents compounds is fascinating. Use of antibacterial medicines over the last 60 years have triggered a combination of genetic and biochemical mechanisms within the bacteria to secure their survival in otherwise lethal environments. Bacterial clones with natural and acquired resistance have continuously been selected as an evolutionary response to the use of antibiotics. Resistance can be acquired as a result of genetic events causing alterations in the pre-existing bacterial genome such as point mutations and gene amplifications. The other major mechanism is horizontal gene transfer between bacteria both within and between species, where transposons, integrons or plasmids are introduced into an organism. The successive introduction of new antibiotics has catalysed the accumulation of resistance mechanisms that travel between microbes, creating clones with multiresistant properties.

1. Introduction

The genetic alterations in bacteria cause resistance to antibiotics in one or more of four principle ways, as shown in Fig. 1.

Bacterial resistance can be defined either genotypically – the bacteria carry certain resistance elements; phenotypically – the bacteria can survive and grow above a certain level of antibiotic in the laboratory; or clinically – the bacteria are able to multiply in humans in the presence of drug concentrations achievable during therapy [5].

2. Are we running out of targets?

Antibiotics are traditionally defined as natural compounds, produced by microorganisms, with selective antibacterial activity that does not have any strong effects on human cells. Their mechanism of action is either through killing the bacteria (bactericidal effect) or by inhibiting bacterial growth (bacteriostatic effect). With the advent of synthetic antibacterial drugs the term antimicrobial agents was initially used to include both synthetic and natural compounds, but as the concept of antibiotics had already become so well established this term took over and is now generally used to include all antibacterial agents.

The introduction of penicillin paved the way for the exploration of various natural compounds, with different targets in the bacterial cell. Penicillin attacks bacteria by inhibiting the cell wall biosynthesis, making the cell wall a weak spot and causing cell lysis. Other substances target different sites within the bacteria and have various effects including inhibition of DNA replication, RNA synthesis and protein synthesis (Fig. 2).

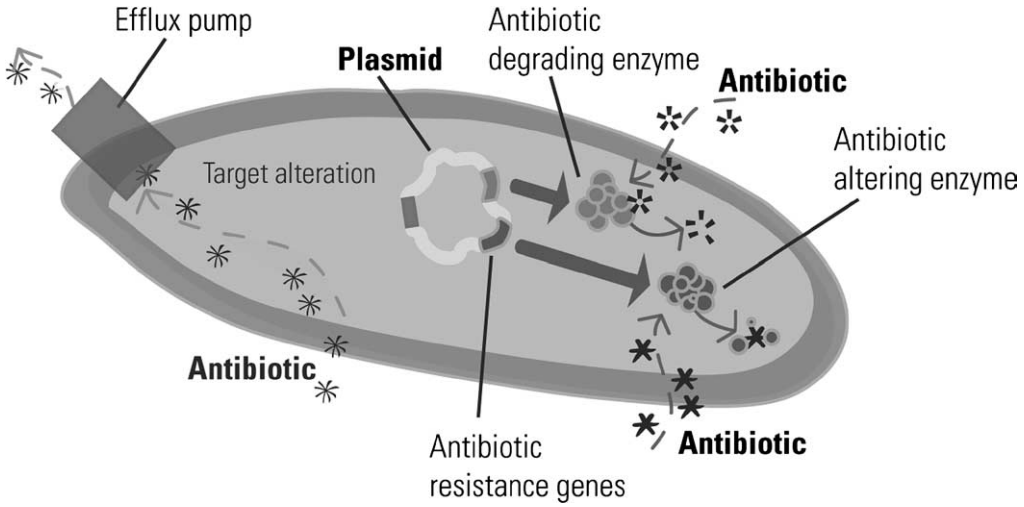

Fig. 1. Target molecules are structurally altered to prevent antibiotic binding; antibiotics are excluded from cell entry; they are inactivated, through enzymatic degradation, for example; or they are or pumped out the cell (efflux).

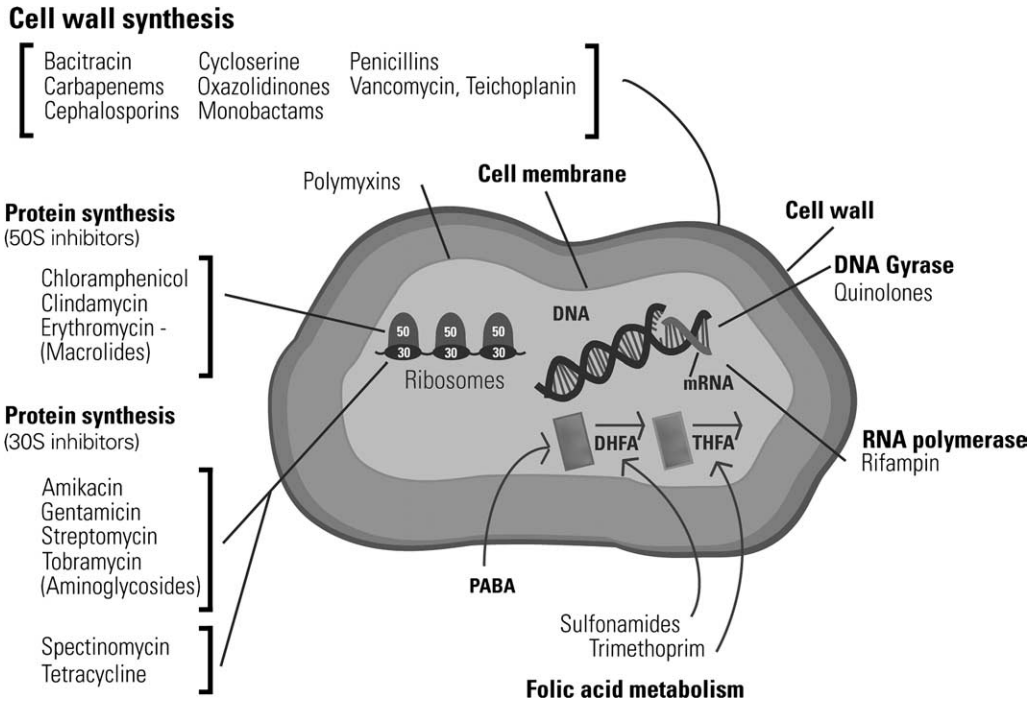

Fig. 2. Bacterial targets for current antibiotics used in the clinic.

Innovative research to find new antibiotic classes subsided in the 1970s, and the focus moved to the refining of existing antibiotic classes. Although such developments have been important to improve the drugs' pharmacokinetics and pharmacodynamics, these modified antibiotics are basically using the same mechanism to attack bacteria as the preceding ones, making it easy for bacteria to develop resistance to the drugs. The promising possibilities that arrived with the novel genomic techniques have so far come

to very little; to our knowledge no drug is yet on market that has been developed through these newer techniques. However, it should be noted that the development time for new antibiotics is 10–12 years and most of these techniques were introduced in pharmaceutical companies in the 1990s. It is also true that the old target sites have not been fully explored; that is, we could most likely find new classes of antibiotics directed at the old targets, such as cell wall biosynthesis, DNA replication, RNA synthesis and protein synthesis. This is illustrated by the fact that the oxazolidinones, the only new class of antibiotics to have been introduced during the last 30 years, are directed at the protein synthesis machinery. This raises the important question of whether our focus should be on finding new potential targets or if we in fact could successfully use the 'old' targets to find the new antibiotics. The latter approach would certainly save us time.

3. Factors driving resistance development

The rate and extent of resistance development depends on a number of factors, some of which are outside our direct control, but some of which we can influence through measures to alter human behaviour, such as improving medical procedures, rationalising prescription practices, influencing consumers' perceptions and expectations, and enforcing restrictions on antibiotic use in animal farming [11,12]. One major controllable factor is the volume of use of antibiotics as this will set the overall selection pressure for resistance development [3,4,9,10]. As a rule it is observed that the frequency of resistance both at the community and the individual level is correlated to antibiotic use. Determining the relative risk of resistance development in the case of any specific antibiotic or dosage regimen is complicated. Influential factors are the antibacterial spectrum of the drug and its pharmacokinetics, such as the building up of concentrations in the gastrointestinal tract, skin and saliva. These factors will influence the extent of impact on the normal flora [17]. Poor patient compliance with dosage regimens and the use of substandard antibiotics lead to intermittent suboptimal serum concentrations that fail to control bacterial populations and are potentially a risk factor for the development of resistance. Experimental studies indicate that certain concentrations may prevent outgrowth of existing resistant bacterial subpopulations. However, at present we lack the detailed knowledge on the pharmacokinetic/pharmacodynamic relationships required to use antibiotics in an optimal manner that minimizes resistance development. This is partly because both the pharmaceutical industry and the regulatory bodies have considered resistance less important than efficacy and safety. Another large knowledge gap is the optimal treatment time, which is unknown for many common bacterial diseases. Recently, large studies in Pakistan and India [7,14] have shown efficacy with a three-day course for pneumonia, and a few studies have indicated that shorter treatment times may lessen the risk of resistance development.

One important determinant of the rate of resistance development that can be influenced by changed human behaviour is infection control: that is, any procedure we can impose that influences the rate of transmission of resistant and susceptible bacteria. Spread of resistance is exacerbated by a number of aspects in modern society, including international trade, ecosystem disturbances, urbanisation and the increasing number of people with compromised immune systems. Infection control measures will reduce the rate of transmission of bacteria; among these are hygienic measures, vaccines, the identification and isolation of patients infected with resistant bacteria, adjusted treatment for these patients and decreased density of patients in clinical wards. Among the factors over which we have no direct control, bacterial mutations and transfer rates of genome through plasmids will have an impact on resistance development by determining how rapidly the resistant variants appear in a bacterial population.

Another uncontrollable factor that has a major influence on resistance development is the fitness cost of resistance combined with the ability of the resistant bacteria to compensate for these costs. Thus, it is often observed that antibiotic resistance exacts a fitness cost of the bacteria, causing them to grow more slowly, become less virulent (for example, in their ability to cause infection) and less transmissible [1,2]. Furthermore, these fitness costs can be reduced or completely eliminated as a result of additional genetic changes, and therefore not lead to loss of resistance. Such compensatory evolution is potentially of great concern since it will cause a stabilization of the resistant bacteria in the population.

Bacteria behave differently when they are continuously exposed to antibiotics, and the rate and extent to which resistance develops are strongly dependent on the particular combination of bacterial species and type of drug. A few years after the introduction of penicillin, reports from British hospitals indicated an almost 50% prevalence of resistant *Staphylococcus aureus*. In contrast, despite of an extensive use of penicillins for the treatment of infections caused by *Streptococcus pyogenes* there has still not been a single case of a penicillin-resistant strain found in a clinical setting. These cases are two extremes of fast and slow resistance development. Importantly, it is generally seen for most combinations of drugs and bacteria that the frequency of resistance at the population level increases both with the length and volume of use and the absence of resistance development. The example of *S. pyogenes* mentioned above is certainly unusual and is not a typical case. It is obviously of great interest to understand why rates of resistance development differ so much [12].

4. Consequences of antibiotic resistance for the individual

When a patient receives treatment with antibiotics, both the causative pathogen and the normal non-pathogenic microflora in the body will be affected. The indigenous microflora make up a complex ecological system of great importance for human health. Besides being essential for the digestion of food and to metabolise drugs, they also produce essential vitamins and are important for the activation and maintenance of the immune system in the gut. Ideally, antibiotics should effectively kill the pathogen responsible for infections and, simultaneously, cause as little disturbance as possible to the microflora of the individual. Presently, the ideal antibiotics do not exist and the overuse of broad spectrum agents in respiratory infections and diarrhoeal diseases consequently drives resistance development in pathogenic bacteria as well as in the normal bacterial reservoir of the patient. This makes them potential carriers of resistant microbes that might be dangerous to themselves and to other patients. The reservoir for resistance mechanisms in the gut can be transferred to more virulent pathogens passing through the body and be spread to other individuals. Furthermore, the bacteria that are carrying resistance mechanisms will be lost very slowly, if at all. Thus, they will form a large reservoir of bacteria that continuously disseminates resistance genes to other microbes. Such long-term persistence of resistant commensal normal flora and the resulting spread of resistance to other bacterial species, including pathogens, have been demonstrated recently. For example, one study showed that when patients were antibiotic-treated for one week for stomach ulcer caused by *Helicobacter pylori* infections, enterococcal bacteria that are part of the normal microflora of the intestine developed high levels of resistance [16]. Importantly, these resistant enterococci remained in the intestine of the treated individuals for up to three years after treatment was finished. Furthermore, in the interconnected world of today, with increased travel and migration, we humans will in a sense assist the bacteria to obtain novel mechanisms for resisting antibiotics all over the world.

5. Reversing resistance?

In the case of both of the major genetic processes, mutations or horizontal gene transfer, the resistant microbe is affected not only in its ability to withstand the antibiotic, but potentially also in its interaction with the host and its ability to be transmitted between hosts. It is generally observed that most resistance mechanisms will confer a reduction in bacterial fitness, which might be expressed as reduced growth and survival inside and outside a host, and reduced virulence or transmission rate from environment to host or between hosts. The observation that resistance is associated with a biological cost has led to the widespread idea that by reducing the volume of antibiotic use the frequency of resistant bacteria in a population can also be reduced. However, this picture is complicated by the fact that bacteria may reduce the costs associated with the resistance through compensatory evolution [1,2,13]. The role of compensatory mutations that maintain the fitness of resistant strains is now well established and increasing levels of biologically competitive resistant bacteria are detected in the community, with no decrease in vitality compared to non-resistant strains. Thus, even in environments where antibiotic pressure is absent, these bacteria may be difficult to remove.

Clinical evidence supporting the reversibility idea is weak. Two epidemiological studies, of erythromycin resistance in *S. pyogenes* [15] and penicillin resistance in *Streptococcus pneumoniae* [3], have been suggested as providing support for the reversibility of resistance in community settings. In these cases, the rate and extent of the decline in the frequency of resistance associated with reduced antibiotic use were small, which is in accord with predictions from modelling. In addition, the weak apparent correlation between reduced antibiotic consumption and decreased frequency of resistance could have been caused by many other factors, for example, clonal shifts where a susceptible clone happened by chance to increase in frequency coincidentally with the reduction in antibiotic use. Thus, the epidemiological studies that are available at the moment provide no strong support for reversibility. In addition, several laboratory and epidemiological studies indicate that various processes are predicted to cause long-term persistence of resistant bacteria. One process is compensatory evolution, where the costs of resistance are ameliorated by additional genetic changes, resulting in the stabilization of resistant bacteria in the population. Even though most resistance is associated with fitness cost, some resistance mutations appear to be gratuitous. The occurrence of such cost-free resistances will also cause irreversibility since the driving force for reversibility is absent. Finally, genetic hitch-hiking between non-selected and selected resistances will confer stabilization of the resistant bacteria. Thus, when two resistance genes are located near each other, on, for example, a plasmid, they tend to be inherited together. As a result, selection for one of the resistance genes tends to cause selection also for the nearby, genetically linked gene. An interesting example of such hitch-hiking was provided by a recent study of sulphonamide-resistant *E. coli*. Here, it was demonstrated that even after a drastic reduction in the use of sulphonamide in the United Kingdom from 1991 to 1999 the frequency of sulphonamide-resistant *E. coli* did not decrease, but actually increased slightly, from 40% to 46%. The explanation for this finding is most likely that the sulphonamide-resistant gene(s) is genetically linked on a plasmid to other resistance genes that were continuously selected during this time period [6]. In conclusion, if antibiotic resistant bacteria have ascended to a high frequency within the community they are likely to remain there for a long time.

In hospital settings the rate and extent of reversibility are much higher than in communities, as shown by both actual experiments and clinical intervention studies as well as by theoretical models [8]. The reason for this difference is that the main driving force for reversibility in hospitals, in contrast to communities, is not the biological cost of resistance. Instead, in hospitals we observe a dilution effect as incoming patients, whether infected or not infected, are in most cases bringing susceptible bacteria into

clinical wards and therefore affect the levels of resistant bacteria. Thus, we predict that reversibility can occur in hospitals in response to reduced antibiotic use as long as the frequency of resistance is lower in the community than it is in the hospital.

6. Beyond the point of no return?

Bacteria will for certain continue to adapt rapidly to new antibiotic substances. Bacterial genomes are not fixed entities as they acquire resistance genes from integrons, transposons and plasmids. Resistance mechanisms are easily exchanged between different species as bacteria in their sophisticated ways share mechanisms essential for survival. Where are we today on the time axis? Antibiotic therapy has existed for 60 years and the frequency of resistant bacteria continues to increase exponentially and makes drugs useless in treating infections. Much evidence supports the idea that resistance among bacteria is irreversible. Can the tide be turned, or are we already beyond the point of no return in the build-up of antibiotic-resistant strains?

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