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Mini review

Antibiotics and antibiotic resistance: A bitter fight against evolution

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ABSTRACT

One of the most terrible consequences of Darwinian evolution is arguably the emergence and spread of antibiotic resistance, which is becoming a serious menace to modern societies. While spontaneous mutation, recombination and horizontal gene transfer are recognized as the main causes of this notorious phenomenon; recent research has raised awareness that sub-lethal concentrations of antibiotics can also foster resistance as an undesirable side-effect. They can produce genetic changes by different ways, including a raise of free radicals within the cell, induction of error-prone DNA-polymerases mediated by SOS response, imbalanced nucleotide metabolism or affect directly DNA. In addition to certain environmental conditions, subinhibitory concentrations of antimicrobials may increase, even more, the mutagenic effect of antibiotics. Here, we review the state of knowledge on antibiotics as promoters of antibiotic resistance.

Introduction

"*... the history of evolution is that life escapes all barriers. Life breaks free. Life expands to new territories. Painfully, perhaps even dangerously. But life finds a way*"
Dr. Malcom in Jurassic Park (Michael Crichton)

During the last 3.5–3.7 billion years, life has got over all the challenges it suffered due to its extraordinary capacity to change and adapt. Microbes, the most extended form of life in our planet have demonstrated a great flexibility and can be found in all environments where they were looked for: from perpetual ices to boiling waters, from extreme pHs to overwhelming pressures. Their high population numbers, their amazing genomic plasticity and their capacity to exchange genetic information among very different species, provide them an endless adaptability. Thus, it should not be strange to us that microorganisms developed mechanisms to resist any weapon that humans develop against them.

It has become clear that the intensive and extended use and misuse of antibiotics in human and veterinary medicine and in agriculture have provoked the worldwide enrichment and spread of highly resistant pathogenic bacteria (Aarestrup, 2005; Cabello, 2006; McManus et al., 2002; Witte, 1998).

In this review, we would try to convince the reader that development and spread of bacterial antimicrobial resistance is just the result of an evolutionary process, i.e. microorganisms adapt to antibiotics as easily as they adapt to a new environmental change. Humans and their products are a tiny part of the history of microbial life, in which antimicrobials are only one more of the challenges that bacteria have to face to survive on Earth. Even more, recent discoveries prompted us to think that bacteria are not mere spectators of their success in adaptation. They can use genetic mechanisms to accelerate or increase the rate of adaptation and take advantage of the adversity (Blazquez et al., 2012; Foster, 1994; Rosenberg, 2001).

Bacteria can evolve antibiotic resistance through several mechanisms, including alteration by mutations of the antibiotic target, changes in cell permeability and efflux, and horizontal transfer of resistance genes (Andersson, 2003; Davies, 1994; Livermore, 2003).

A major breakthrough in understanding antibiotic resistance evolution came with the demonstration in the 40s that the exposure of bacteria to lethal agents results in the selection of pre-existing resistant variants and, therefore, selecting agents do not induce the appearance of resistant strains (Lederberg and Lederberg, 1952; Luria and Delbruck, 1943; Newcombe, 1949). However, after seven decades of research, we have to expand this classical view to reflect the current understanding this complex phenomenon.

For instance, what happens when antibiotics are present at low, even very low, concentrations (not sufficient to kill or stop the growth of the susceptible population) as those present in many environments?

Antibiotics do more than select for resistant clones

Subpopulations of bacteria can survive lethal doses of antibiotics without becoming resistant by a transient and non-hereditary mechanism, called persistence. A recent work (Dorr et al., 2009) shows that a majority of persisters to the quinolone ciprofloxacin appeared upon exposure to the antibiotic in an SOS-dependent

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manner. Therefore, persisters are formed by an active and inducible mechanism mediated by the SOS response, which is induced by some antibiotics. This contrasts with the previous view that persisters appear by stochastic means previously to the antimicrobial challenge.

Antibiotic pressure also selects for bacteria with an elevated mutation rate (hypermutators or mutators). Since these alleles increase the possibility of favourable mutations, they can accelerate the evolutionary rate under some conditions. During this process, mutator bacteria can be fixed in the population by a second order selection, 'hitchhiking', with the favourable mutations they have originated (Taddei et al., 1997). This heritable hypermutation in bacteria is mainly produced by alterations in genes belonging to the mismatch repair (MMR) system (*mutS*, *mutL*, *mutH* and *uvrD*) (Oliver et al., 2000, 2002; Radman et al., 2000), although deficiency in other antimutator genes, such as *mutT* (which cleans 8-oxo-G and 8-oxo-dG), *mutY* and *mutM* (able to eliminate the mistakes produced by the incorporation of 8-oxo-dG into DNA) have also been found among mutator strains of *Pseudomonas aeruginosa* in cystic fibrosis patients (Ciofu et al., 2009). The lack of the MMR system increases, not only the mutation rate, but also the frequency at which two divergent sequences, coming from the same or different bacterial species, recombine (Rayssiguier et al., 1989). Thus, the probability of acquiring new functions by both mutation and recombination is greatly favoured in MMR-deficient strains. Mao et al. (1997) demonstrated that antibiotic pressure can select for mutators. Single selection for a mutant resistant to an antibiotic increases the proportion of mutators in the selected population from the original 0.001% (the normal frequency in an *E. coli* population) to as much as 0.5%. Furthermore, successive selections could increase the proportion of mutator strains in the selected population to 100% (Mao et al., 1997). Therefore, a given antibiotic might not only select for resistance to itself, but may also, by increasing the proportion of mutators, indirectly select for the increased probability of resistance to non-related antibiotics.

Antibiotics cause direct mutagenic effects

Several antibiotics can increase the mutation rate in different ways, including oxidative damage (Kohanski et al., 2007), SOS response (Baharoglu and Mazel, 2011; Blazquez et al., 2006; Gocke, 1991; Miller et al., 2004; Perez-Capilla et al., 2005; Thi et al., 2011), nucleotide-pool unbalancing (Genther et al., 1977), and general stress responses (for a review see Foster, 2007).

The production of reactive oxygen species (ROS) has been postulated as a common step in antibiotic mediated lethality (Dwyer et al., 2009). This common pathway to cell death is mediated by an increased respiration rate, a transient depletion of NADH and the irreversible oxidation of the iron–sulfur clusters, which lead to hydroxyl radical generation via Fenton reactions (Dwyer et al., 2009; Kohanski et al., 2007). However, this mechanism seems to be not strictly necessary, since quinolones and cephalosporins are equally effective in anaerobic conditions. ROS are known to cause damage in key cellular components such as proteins, lipids and DNA. This damage can cause DNA lesions either directly or indirectly, which if not repaired, lead to the accumulation of mutations. Treatment of *E. coli* with some antibiotics at sublethal concentrations increases ROS levels, which correlate significantly with an increase in mutagenesis (Kohanski et al., 2010).

As a consequence of the ROS increase or replication fork stall (produced, for instance by fluoroquinolones), or both, the SOS response is activated. Several functionally unrelated antibiotics induce the SOS system (Kohanski et al., 2007; Thi et al., 2011). This activation triggers the expression of specialized (error-prone) DNA-polymerases able to bypass DNA lesions with reduced fidelity (Jarosz et al., 2007). In fact, it is known that quinolones are mutagenic in bacteria (Gocke, 1991). This is why subinhibitory concentrations of quinolones may increase the frequency of resistance mutations. Ciprofloxacin produces an increase of up to 5-fold in the frequency of rifampin resistant mutants in *Streptococcus pneumoniae* (Henderson-Begg et al., 2006) and carbapenem resistant variants in *P. aeruginosa* (Tanimoto et al., 2008). In *Mycobacterium fortuitum*, the same antimicrobial produces an impressive rise in mutant frequency to nearly two orders of magnitude (Gillespie et al., 2005). In *E. coli*, in vitro inactivation of the *recA* gene, which is required for the induction of the SOS response, counteracts the effect of sublethal antimicrobial concentrations on mutagenicity (Thi et al., 2011). Additional regulators involved in stress-induced mutagenesis in *E. coli* are the sigma factors RpoS and RpoE (Frisch et al., 2010; Gibson et al., 2010), which may exert an independent inactivation of some error-prone DNA-polymerases.

β-Lactam antibiotics, such as penicillins and cephalosporins, also induce the SOS response but do so via a completely different pathway (Miller et al., 2004). Inhibition of cell division by exposure to β-lactams induces the *dpiBA* operon, which encodes a response-effector two-component system. DpiA, the effector, binds to the chromosomal replication origin and inhibits replication, inducing the SOS response and, increasing genetic variability (Miller et al., 2004). Exposure to β-lactam antibiotics induces the *dinB* gene and mutagenesis also via an SOS-independent pathway (Perez-Capilla et al., 2005). On the other hand, the mechanism of trimethoprim-induced mutation has been attributed to nucleotide pool imbalance (Genther et al., 1977) because DNA-polymerases replicate with reduced fidelity when face nucleotide pool imbalance. Moreover, sublethal doses of streptomycin result in inaccurate translation (Balashov and Humayun, 2003; Rosset and Gorini, 1969) and mistranslation of DNA repair and replication proteins, creating transient mutator states (Ninio, 1991). Interestingly, mistranslated proteins after streptomycin treatment were shown to be more susceptible to oxidation by ROS (Dukan et al., 2000).

In *E. coli*, fluoroquinolones, β-lactams, trimethoprim and sulfamethoxazol induce the SOS stress response, whereas aminoglycosides, tetracycline and chloramphenicol, do not (Li et al., 2008a,b). However, these antibiotics also induce SOS in *Vibrio cholerae* (Baharoglu and Mazel, 2011).Furthermore, subinhibitory levels of antibiotics may stimulate not only bacterial mutation but also recombination (see below) (Lopez and Blazquez, 2009; Lopez et al., 2007). Therefore, human body and environmental sites exposed to low concentrations of antimicrobials may become antibiotic-induced mutation and recombination hotspots, responsible for phenotypic variation and specifically for the emergence, maintenance and dissemination of antibiotic resistance.

According to the above data, RecA and LexA (the master regulators of the SOS response) have been proposed as targets to prevent or reduce the appearance of resistance mutants during antibiotic treatments (Cirz et al., 2005; Wigle et al., 2009).

Effects of antibiotics on horizontal gene transfer and recombination

In addition to mutation, there are other mechanisms that generate genetic variation in bacteria, such as intragenomic reorganization of genomic sequences (intrachromosomal recombination) and the acquisition of foreign DNA sequences from other organisms by means of horizontal gene transfer (HGT). Both mechanisms play an important role in bacterial evolution and adaptation, including evasion of the immune response, distribution of genes that increase virulence and increasing resistance to antibiotics (de la Cruz and Davies, 2000; Guttman and Dykhuizen, 1994; Lawrence and Ochman, 1998; Lawrence and Roth, 1996). The majority of

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