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Minireview

The role of antibiotics and antibiotic resistance in nature

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Summary

Investigations of antibiotic resistance from an environmental prospective shed new light on a problem that was traditionally confined to a subset of clinically relevant antibiotic-resistant bacterial pathogens. It is clear that the environmental microbiota, even in apparently antibiotic-free environments, possess an enormous number and diversity of antibiotic resistance genes, some of which are very similar to the genes circulating in pathogenic microbiota. It is difficult to explain the role of antibiotics and antibiotic resistance in natural environments from an anthropocentric point of view, which is focused on clinical aspects such as the efficiency of antibiotics in clearing infections and pathogens that are resistant to antibiotic treatment. A broader overview of the role of antibiotics and antibiotic resistance in nature from the evolutionary and ecological prospective suggests that antibiotics have evolved as another way of intra- and inter-domain communication in various ecosystems. This signalling by non-clinical concentrations of antibiotics in the environment results in adaptive phenotypic and genotypic responses of microbiota and other members of the community. Understanding the complex picture of evolution and ecology of antibiotics and antibiotic resistance may help to understand the processes leading to the emergence and dissemination of antibiotic resistance and also help to control it, at least in relation to the newer antibiotics now entering clinical practice.

Introduction

Antibiotics are probably one of the most successful forms of chemotherapy in the history of medicine. They have saved many millions of lives and placed the majority of infectious diseases that plagued human history for many centuries under control. Initially, on their introduction into clinical practice in the 1940s, antibiotics were extremely efficient in clearing pathogenic bacteria leading many to believe that infectious diseases would become a problem of the past and would be wiped out from all human populations eventually. However, the emergence and rapid dissemination of antibiotic-resistant pathogens, especially multi-drug-resistant bacteria, during recent decades, exposed our lack of knowledge about the evolutionary and ecological processes taking place in microbial ecosystems. It is now evident that microbial populations possess enormous metabolic diversity, from which they may deploy protective mechanisms allowing them to withstand the selective pressures imposed by their natural environment as well as human interventions such as antibiotics. Revealing the nature and functional role played by antibiotics and antibiotic resistance in various natural ecosystems may help to understand the processes leading to the emergence of antibiotic-resistant pathogens. Finally, armed with knowledge accumulated through many years of genetic, genomic and metagenomic studies and with new concepts about antibiotics and antibiotic resistance, can we now predict the emergence and dissemination of resistance to newly introduced antibiotics?

This review will focus mainly on areas that have contributed to re-evaluation of our conceptual framework about antibiotics and the problem of antibiotic resistance. In particular, the contribution of evolutionary, ecological and functional aspects of antibiotics and antibiotic resistance will be reviewed. In the final section, the practical implications of an attempt to apply these new concepts to the prediction of resistance to a novel antibiotic will be made.

Evolution of antibiotic resistance genes

On an evolutionary scale, the massive explosion of antibiotic-resistant phenotypes in human and animal pathogens is a very recent event that has followed the large-scale production and use of antibiotics in clinical and veterinary medicine, agriculture, aquaculture,

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horticulture and other human activities. It was thought, initially, that the genetic variability of material in bacterial populations for selection by antibiotics to operate would be through mutations and, as a such, antibiotic resistance would be based largely on target modification and so remain clonal. Indeed, this mechanism is still dominant in the case of resistance to quinolones, rifampin and fosfomycin and it drives the structural evolution of horizontally transferred antibiotic resistance genes such as extended-spectrum beta-lactamases (ESBLs). Mutation-driven antibiotic resistance, however, happens mainly during in-host evolution such as in chronic infections (Maciá *et al*., 2005) while the purpose of this review is to discuss antibiotic resistance from a broader environmental prospective. The vast majority of antibiotic resistance mechanisms are acquired through horizontal gene transfer from other, often taxonomically very distant, bacteria. Phylogenetic analysis of several groups of antibiotic resistance genes has suggested that genetic material for present-day antibiotic resistance has had a long history of selection and diversification well before the current 'antibiotic era' (Aminov and Mackie, 2007).

Probably the best-documented case of the ancient evolution of antibiotic resistance genes comes from the analysis of β-lactamases. β-Lactams are the most widely used antibiotics in clinical medicine and resistance to β-lactams may become a severe threat because they have low toxicity and are used to treat a broad range of infections (Livermore, 1996). The primary resistance mechanism is enzymatic inactivation through the cleavage of the β-lactam ring by β-lactamases. These enzymes are represented by two unrelated groups, one comprising of three classes of serine β-lactamases, with an active-site serine, and another – of two classes of metallo-β-lactamases, which require a bivalent metal ion to catalyse the hydrolysis (Bush, 1998). Both groups are very ancient and the classes within the groups are diversified to the extent that all traces of homology between the classes at the sequence level are lost (Hall and Barlow, 2004; Garau *et al*., 2005). Structure-based phylogeny was, however, able to reconstruct the evolution of both β-lactamase groups and establish that these ancient enzymes originated more than two billion years ago, with some serine β-lactamases being present on plasmids for millions of years, well before the modern use of antibiotics (Hall and Barlow, 2004; Garau *et al*., 2005). Recent work on the evolutionary history of β-lactamase genes in *Klebsiella oxytoca* has suggested that these genes have been evolving for over 100 million years in this host, without concomitant evolution of the antimicrobial resistance phenotype and with the phylogenies of β-lactamase and housekeeping genes being highly congruent in this organism (Fevre *et al*., 2005). Molecular analysis of β-lactamases in a metagenomic library from 'cold-seep' sediments also showed that most of the diversity of these enzymes is not the result of recent evolution, but is that of ancient evolution (Song *et al*., 2005). Our own, limited, phylogenetic analysis of class A β-lactamases, with the inclusion of sequences from antibiotic producers such as *Amycolatopsis lactamdurans* and streptomycetes as well as from the environmental bacteria, essentially confirmed these findings (data not shown). Interestingly, the unknown evolutionary forces in apparently antibiotic-free environments may also contribute to the generation of novel diversity in antibiotic resistance genes (Allen *et al*., 2009). This metagenomic study of Alaskan soil not only uncovered a diverse and ancient collection of β-lactamase genes, but also revealed a novel gene encoding a bifunctional β-lactamase that has never been encountered before.

Recently, we subjected several groups of antibiotic resistance genes conferring resistance to structurally unrelated antibiotics, with different mechanisms of action, to rigorous phylogenetic analyses (Aminov and Mackie, 2007). In general, the history of antibiotic resistance genes can be divided into the macro- and microevolutionary periods, which can also be defined as the 'pre-antibiotic' and 'antibiotic' periods. The former is characterized by a long history of diversification in natural ecosystems, mostly through duplications and mutations, with a limited contribution of horizontal gene transfer to the processes. What is not known is if these processes have been involved in providing an antibiotic resistance function *per se* or might have served some other metabolic function. The antibiotic era, in fact, was (and still is) a fairly brief evolutionary experimentation that was conducted during the past 70 years with large-scale production of antibiotics and exertion of a strong selective pressure towards bacteria in various ecosystems. Relatively rare genes that happened to confer antibiotic resistance were once involved in other cellular functions but were selected for the resistance phenotype and mobilized from the environmental genomic reservoirs, with the rapid dissemination into taxonomically divergent commensal and pathogenic bacteria. The process was very rapid on an evolutionary scale and horizontal gene transfer, mediated by mobile genetic elements, played a prominent role in it.

Based on the historical evolutionary background, the next question to ask is what possible functional role is played by antibiotics and antibiotic resistance in natural ecosystems?

Ecology of antibiotics and antibiotic resistance

The question of how antibiotic resistance genes are maintained in the environment is contradictory. If the sole functional role of these genes is to confer protection against the lethal concentrations of antibiotics, then selection and

dissemination of antibiotic resistance genes should be linked to anthropogenic factors since environmental concentrations of antibiotics in unaffected areas are normally below detection limits and certainly not in the minimum inhibitory concentration (MIC) range for the majority of environmental bacteria. Indeed, this assumption may be supported by cases of detectable antibiotic resistance gene flow from facilities that use antibiotics into the environment (Chee-Sanford *et al*., 2001; 2009; Koike *et al*., 2007; Baquero *et al*., 2008). On the other hand, there are many cases of persistence of antibiotic resistance genes in apparently antibiotic-free environments. In the metagenomic studies of 'cold-seep' sediments and remote Alaskan soil, the presence of diverse β-lactamase genes in both locations has been demonstrated (Song *et al*., 2005; Allen *et al*., 2009). Judging by the total amount of environmental DNA sampled and the number of β-lactam-resistant clone encountered, about 5% of average-sized bacterial genomes in this type of undisturbed soil may carry the β-lactamase genes that are readily expressed in *Escherichia coli*. In another example of antibiotic resistance in unaffected environments, the phenotype of more than 60% of the *Enterobacteriaceae* isolates from the pristine freshwater environment was found to be multi-drug-resistant (Lima-Bittencourt *et al*., 2007).

It is now generally recognized that the natural environment harbours a vast diversity of antibiotic resistance genes and some soil bacteria may even subsist on antibiotics using them as their sole source of carbon (D'Costa *et al*., 2006; 2007; Wright, 2007; Martínez, 2008; Dantas *et al*., 2008). What, then, is the functional role played by these genes in the environment? The term 'antibiotics' is commonly referred to their therapeutic use and the ultimate goal of any antibiotic therapy is clearing up an infection. Is the same role, in a Darwinian natural selection sense, played by antibiotics and antibiotic resistance in the environment? Indeed, certain strains of fluorescent pseudomonads colonizing euthrophic econiches such as the rhizosphere may suppress soil-born pathogens through the production of diffusible or volatile antibiotics such as phenazines, phloroglucinols, pyoluteorin, pyrrolnitrin, cyclic lipopeptides and hydrogen cyanide (Haas and Défago, 2005). At the same time, several lines of evidence collected in recent years indicate that antibiotic concentrations occurring in natural oligothrophic environments may be too low to exert any lethal effects and, instead, they may play signalling and regulatory roles in microbial communities (Davies *et al*., 2006; Linares *et al*., 2006; Yim *et al*., 2006; Martínez, 2008). If antibiotics are indeed involved in signalling, then what kind of signals they convey? If the signals are related to the environmental conditions, physiological state or other regulatory networks? The most appropriate models to discuss these questions would be the antibiotic producers and regulation of antibiotic synthesis in these bacteria.

Antibiotics as yet another language of communication

The vast majority of commercially available antibiotics are produced by *Streptomyces* spp. (Weber *et al*., 2003) and the pioneering studies of antibiotic synthesis regulation in the representatives of this genus has resulted in identification of a γ-butyrolactone or A-factor that induces antibiotic production and differentiation in *Streptomyces griseus* (Khokhlov *et al*., 1967). A pathway for A-factor biosynthesis has been recently proposed (Kato *et al*., 2007). This group of molecules, represented by three major types, namely 6-keto (6*R*)-hydroxy and (6*S*)-hydroxy types (Nishida *et al*., 2007), belongs to the quorum-sensing (QS) system, the best-studied prototype of which is the *Vibrio fischeri* QS network (Fuqua *et al*., 1996). The QS is widespread among bacteria and serves as a language of communication, not only between the bacteria but also in inter-kingdom signalling (Shiner *et al*., 2005). Similarly to the LuxI/LuxR system of Gram-negative bacteria, the γ-butyrolactone signalling system in *Streptomyces* consists of the γ-butyrolactone synthase, AfsA, and the receptor protein, ArpA (Nishida *et al*., 2007). During growth, γ-butyrolactones are gradually accumulated in the media and, when they reach critical concentrations, interact with the DNA-binding cytoplasmic receptor proteins, ArpA and its homologues, releasing them and allowing transcription from target genes. The target genes are transcriptional factors that are involved in the production of secondary metabolites such as antibiotics and/or in morphological differentiation (Horinouchi, 2007).

The availability of many sequenced bacterial genomes allowed to search for proteins with homology to γ-butyrolactone synthases and receptors. Interestingly, only 10 or 11 probable γ-butyrolactone synthases, all from the representatives of the *Streptomyces* genus, were found while 37–42 putative γ-butyrolactone receptors were found in genomes of bacteria not only from the *Streptomyces* but also from *Kitasatospora*, *Brevibacterium*, *Saccharopolyspora*, *Mycobacterium*, *Rhodococcus*, *Anabaena*, *Nocardia* and *Nostoc* genera (Takano, 2006; Nishida *et al*., 2007). Thus the behaviour of bacteria in a community may be orchestrated through a small number of γ-butyrolactone producers, with a much larger and diverse audience of signal receivers. Consistent with this, the evolution of γ-butyrolactone synthases and its receptors was not congruent (Nishida *et al*., 2007). Besides, the ancestral receptors, initially, functioned as regulatory DNA-binding proteins and only later in evolution acquired the γ-butyrolactone-sensing capability (Nishida *et al*., 2007). As this example demonstrates, the well-known QS

signalling network initiates a set of metabolic changes in the community leading to a number of events including differentiation, synthesis of secondary metabolites such as antibiotics and probably other cellular processes.

With few exceptions, such as simple microbiota that occupy extreme ecological niches, functional redundancy is built into many microbial ecosystems to ensure homeostasis and continuous operation even in the case of an acute external stress. The signalling systems in various ecosystems also have a similar level of redundancy, through multiple regulatory networks. The QS system represents one such signalling network and its role in intra- and inter-species communication, as well as in regulation of many aspects of metabolism, virulence, physiology, competence, motility, symbiosis and other functions is described in many excellent reviews. There are a number of other regulatory networks such as two-component systems and various sensors that convey the environmental information to the cell. The languages used in this type of communication, however, have many dialects and are quite specific because they require specific receptors for the signals to be correctly perceived, and only a limited number of microbiota members (roughly fourfold bigger than the original signal producers in the case of antibiotic synthesis; see the ratio of γ-butyrolactone synthases and receptors above) may adequately respond to these signals by reorganizing their cellular processes. Is it that the synthesis of antibiotics is also a signalling mechanism? Then what is the function of this mechanism? Is it in amplification of the initial weak and rapidly decaying signal in the environment to make it stronger and less specific so it is perceived by other members of the community that are not capable of sensing and deciphering the environmental signals themselves? In support of this notion, indeed, the acyl-homoserine lactone quorum signal very rapidly decays in many soil types (Wang and Leadbetter, 2005). In this scenario, the function of antibiotic resistance may be in attenuating signal intensity similar to quorum quenching in the QS communication (Dong *et al*., 2001). Indeed, removal of the initial QS signal renders bacteria more sensitive to antibiotics (Ahmed *et al*., 2007). The negative feedback loop of the secondary signalling system, antibiotics, may then suppress the primary QS signalling network (Tateda *et al*., 2004; Skindersoe *et al*., 2008) thus providing the fine-tuning between the two signalling networks.

If antibiotics do indeed play a universal signalling role in natural ecosystems then we would expect to see examples of convergent evolution, for example, in production of the same type of signalling molecules by taxonomically different bacteria employing different biosynthetic pathways. For historical reasons, the search for antibiotic producers was largely confined to streptomycetes (from which the first antibiotics were successfully developed) and, probably because of this, the list of known antibiotic producers is heavily biased towards this group of bacteria. Antibiotic biosynthesis may be a much broader phenomenon in nature and the inclusion of other groups of bacteria and methods of testing in antibiotic screening programmes may uncover the true extent of the environmental antibiome. For instance, β-lactams are a ubiquitous group of antibiotics and are produced by a wide range of bacteria. For the sake of brevity, only the carbapenem group of β-lactams, one of the most therapeutically potent antibiotics currently available (Nicolau, 2008), will be discussed in the context of convergent evolution and signalling and regulation in microbial ecosystems.

The first carbapenem-producing bacterium identified was *Streptomyces cattleya* (Kahan *et al*., 1972). Structurally similar antibiotics were later described in other *Streptomyces* species as well as in Gram-negative bacteria belonging to the *Serratia* and *Erwinia* genera (Parker *et al*., 1982). Carbapenem compounds are also produced by a luminescent entomopathogenic bacterium *Photorhabdus luminescens* (Derzelle *et al*., 2002). Carbapenems are synthesized via a different metabolic pathway from that employed in the classical β-lactam biosynthesis route for penicillins, cephamycins and cephalosporins (Williamson *et al*., 1985). The genes involved in the synthesis of carbapenems are organized into clusters (McGowan *et al*., 1997; Cox *et al*., 1998; Derzelle *et al*., 2002; Núñez *et al*., 2003) and although the biosynthetic pathways share some similar enzymes in Gram-positive and Gram-negative producers, they are substantially different (Coulthurst *et al*., 2005). These enzymes probably evolved from primary metabolic enzymes in corresponding producers and represent an example of convergent evolution.

The regulation of carbapenem biosynthesis in Gram-negative bacteria also represents an interesting example of its dependence on environmental factors, in particular of QS. Despite the structural similarity between the carbapenems from streptomycetes and a range of Gram-negative bacteria, its regulation is governed by QS signals specific for a given group of bacteria. In *Erwinia carotovora* (recently reclassified as *Pectobacterium carotovorum*) its biosynthesis is regulated by a classical autoinducer, *N*-(3-Oxohexanoyl)-L-homoserine lactone (Bainton *et al*., 1992). Despite less dependence on antibiotic production from the growth phase and cell density in *Serratia*, antibiotic production is also under a QS control in these bacteria (Thomson *et al*., 2000). However, a number of other signals are also integrated into the regulatory network in *Serratia* (Slater *et al*., 2003; Fineran *et al*., 2005). The complexity of this regulation may reflect the fine-tuning mechanisms regulating the level of antibiotic production in response to the multiple signals perceived by these bacteria from the environment.

Interestingly, cryptic carbapenem antibiotic production genes are widespread in *E. carotovora* and, in laboratory conditions, this phenotype can be suppressed by multiple copies of the apparently mutant transcriptional activator (Holden *et al*., 1998). It is possible, however, that the antibiotic is synthesized in natural ecosystems but the environmental factors governing its expression are not known. Another possibility might be that if an antibiotic serves as a signalling molecule then the concentrations produced in the environment may be too low to be detected in the MIC and instrumental assays. Much less information is available regarding the regulation of carbapenem biosynthesis in *S. cattleya* but a recent publication suggests that there is an additional, low-level, cross-talk between the thienamycin and cephamycin C pathways in this bacterium (Rodríguez *et al*., 2008). In another cephamycin C-producing bacterium, *Streptomyces clavuligerus*, antibiotic synthesis is regulated at the primary regulatory level by γ-butyrolactone (Liras *et al*., 2008). Therefore, the case with carbapenems also supports the hypothesis that cells respond to the initial QS signals and other environmental clues such as *N*-acetylglucosamine (Rigali *et al*., 2008) and nutrient depletion (Hesketh *et al*., 2007; Lian *et al*., 2008), possibly integrating them, by the second level of signalling, through antibiotics. Continuing with the carbapenems as an example, the second-level signalling by the representative of this class of antibiotics, imipenem, at subinhibitory concentrations, involves changes in global gene expression, including β-lactamase and alginate production, in *Pseudomonas aeruginosa* biofilms (Bagge *et al*., 2004). In Gram-positive bacteria, low-concentration carbapenems are potent inhibitors of L,D-transpeptidases that catalyse the formation of 3→3 peptidoglycan cross-links and bypass the 4→3 cross-links formed by the D,D-transpeptidase activity of penicillin-binding proteins (PBPs) (Mainardi *et al*., 2007; Lavollay *et al*., 2008). In *E. coli*, the L,D-transpeptidase homologue is involved in attachment of the Braun lipoprotein to peptidoglycan (Magnet *et al*., 2007). The lack of the lipoprotein in *E. coli* leads to sensitivity to EDTA, cationic dyes and detergents but no vital cellular functions are affected (Hirota *et al*., 1977). The question is, how other antibiotic signals are perceived and what kind of phenotypic and genotypic responses they may evoke in other systems?

Phenotypic responses to antibiotic signalling

The question about what range of antibiotic concentrations is effective for cross-talk to occur in natural ecosystems remains open. The concentrations of antibiotics that occur in natural, unaffected, environments are unknown and, in a few cases, these environments have actually served as control/zero points for measuring the impact of human activity on natural ecosystems, with no detectable antibiotics present (Yang and Carlson, 2003; Kim and Carlson, 2006; Pei *et al*., 2006). The natural ecosystems sampled were, however, river and sediment environments, which are probably not the most appropriate ecosystems to investigate antibiotic communication. There is a substantial literature, however, describing concentration-dependent bacterial responses to antibiotics in laboratory conditions, which was recently reviewed within the frames of hormesis concept (Davies *et al*., 2006; Fajardo and Martínez, 2008). In this concept, low antibiotic concentration regulates a specific set of genes in target bacteria, while increasingly higher concentrations elicit a stress response and even higher concentrations are lethal. It has been suggested that antibiotics play a role in the environment at low concentrations unlike the lethal concentrations used in clinical therapy.

The subinhibitory antibiotic effects have been studied in a number of bacteria, mostly pathogens and in relation to virulence and pathogenic properties. The best-studied model among them is the opportunistic pathogen *P. aeruginosa*, infection with which is often fatal for cystic fibrosis patients. The central role in regulation of pathogenic properties of *P. aeruginosa* belongs to the QS system and infection with QS-deficient mutants results in less severe outcome in animal models (Girard and Bloemberg, 2008). It has been demonstrated *in vitro* that the subinhibitory levels of a macrolide antibiotic azithromycin suppress one of the pathogenic properties of *P. aeruginosa* as alginate overproduction and biofilm formation (Ichimiya *et al*., 1996). In addition to macrolide antibiotic azithromycin, the subinhibitory concentrations of other antibiotics such as ceftazidime (β-lactam) and ciprofloxacin (fluoroquinolone) also appeared to be effective in decreasing the expression of a range of QS-regulated virulence factors (Skindersoe *et al*., 2008). The authors hypothesized that the effect of antibiotics is due to changes in membrane permeability affecting the flux of *N*-3-oxo-dodecanoyl-L-homoserine lactone. On the other hand, exposure of *P. aeruginosa* to subinhibitory concentrations of another β-lactam, imipenem, results in the increase of alginate production and biofilm volume (Bagge *et al*., 2004). Another fluoroquinolone, norfloxacin, also induces biofilm formation at subinhibitory concentrations (Linares *et al*., 2006). These contradictory results suggest that antibiotics of the same class, which share the same molecular targets to execute their lethal activity, may have different targets in the cell when functioning as signal molecules. Indeed, subinhibitory concentrations of the macrolide-lincosamide-streptogramin antibiotics demonstrate the dual effects, interacting with the ribosomes and modulating transcription (Tsui *et al*., 2004). At the same time, blocking the known target of lethal activity of azithromycin may abolish its QS modulatory properties

(Köhler *et al*., 2007). Since the ribosome is a target for many different antibiotics, then can it serve as a multi-ligand sensor? Protein synthesis is the most expensive biosynthetic activity in the cell; is it because of this the antibiotic signals are aimed at this regulatory target when the resources become scarce? The notion that cellular targets for the lethal and subinhibitory actions of antibiotics may differ is further supported by the effect of subinhibitory concentrations of an aminoglycoside antibiotic tobramycin, which induces biofilm formation in *P. aeruginosa* and *E. coli* (Hoffman *et al*., 2005). In *P. aeruginosa*, molecular target for subinhibitory tobramycin is not the ribosome but aminoglycoside response regulator, an inner-membrane phosphodiesterase whose substrate, cyclic di-guanosine monophosphate, is a bacterial second messenger that regulates cell surface adhesiveness. Identification of targets for other subinhibitory antibiotics therefore remains a priority.

In *Staphylococcus aureus*, virulence is largely regulated by two-component regulatory systems, such as the *agr*, *saeRS*, *srrAB*, *arlSR* and *lytRS* systems (Bronner *et al*., 2004) and regulatory RNA such as RNAIII (Boisset *et al*., 2007). For the sake of brevity, only one of these system, SaeRS, will be discussed in the context of subinhibitory antibiotics. This system is strongly involved in the tight temporal control of virulence factor expression (Rogasch *et al*., 2006) and its inactivation eliminates adherence and attenuates virulence of *S. aureus* in a murine infection model (Liang *et al*., 2006). The sensor molecule of this system, SaeS, is activated by alteration within the membrane allowing the pathogen to react to phagocytosis-related effectors (Geiger *et al*., 2008). Subinhibitory β-lactam concentrations are able to induce the SaeRS system, thus enhancing the virulence of *S. aureus* (Kuroda *et al*. 2007). At 0.5× MIC, florfenicol as well, increases the expression of *saeRS* up to fivefold, with the concomitant increase in the expression of genes coding for adhesins (Blickwede *et al*., 2005a). Interestingly, this ribosome-targeting antibiotic contributes to the stabilization of the respective mRNAs thus suggesting a mechanism for the SaeRS system activation by subinhibitory florfenicol. The mRNA stabilization effect of subinhibitory concentrations is also documented for another ribosome-targeting antibiotic, tetracycline (Wei and Bachhofer, 2002). Although a subinhibitory concentration of clindamycin likewise leads to the stabilization of mRNA of *S. aureus* adhesins this is not reflected in the phenotype (Blickwede *et al*., 2005b).

In general, bacterial transcriptional responses to subinhibitory antibiotics, assessed with microarrays, are not consistent and seem to depend on a variety of factors such as experimental conditions, the nature and concentration of antibiotic used, taxonomy and genotype of bacteria, and others (Davies *et al*., 2006). Also, the extent of transcriptional response does not necessarily mean that it is automatically converted into the corresponding phenotype. Given the complexity of regulatory networks and circuits, with which subinhibitory antibiotics may interact, and the dynamic nature of responses, the number of variables in this type of experiments must be minimized and standardized in order for the results to be comparable.

The details of subinhibitory antibiotics signalling in eukaryotes were mainly uncovered through the observation of antibiotic effects in animal models, tissue cultures, and in clinical trials. Tetracyclines, macrolides and ketolides display potent anti-inflammatory activities (Tamaoki *et al*., 2004; Weinberg, 2005; Del Rosso, 2007; Webster and Del Rosso, 2007; Leiva *et al*., 2008a,b) while rifampin exerts the opposite effect (Yuhas *et al*., 2008). The macrolide antibiotic-binding human p8 protein was recently cloned and identified using the phage display library approach (Morimura *et al*., 2008). This is a nuclear DNA-binding protein, which is strongly activated in response to several stresses, and, on the basis of functional similarity to HMG-I/Y-like proteins, it was suggested that p8 may be involved in transcription regulation (Encinar *et al*., 2001; Hoffmeister *et al*., 2002). Since clarithromycin, erythromycin and azithromycin inhibit the binding of recombinant p8 protein to double-stranded DNA (Morimura *et al*., 2008), the anti-inflammatory effect of macrolides may be explained by the downregulation of transcription of genes involved in the pro-inflammatory network. Interestingly, the same inhibitory effect was observed with a structurally unrelated antifungal antibiotic dechlorogriseofulvin (Morimura *et al*., 2008) suggesting a potential overlap in recognition of structurally different antibiotic ligands by a single human molecular sensor.

These antibiotic activities that are beyond their intended antibacterial use lead to several implications. First, the choice of antibiotics for treatment of infections in patients with chronic inflammatory diseases should take into account potential side-effects such as upregulation of the inflammatory network as in the case with rifampin. Second, antibiotics that have been approved for human and animal use can be screened for clinical activities other than antibacterial, such as functioning as ligands for targets and receptors involved in human and animal disease and pathology. Third, could the immunomodulatory effects of antibiotics be explained by long-term co-evolution of host–microbe cross-talk mechanisms? One of the attempts to explain a dramatic increase in the number of allergies and asthma in more developed countries suggests that these diseases are the result of the limited exposure to the environmental antigens in the modern world (Strachan, 1989). Continuous exposure of humans to environmental bacteria synthesizing a plethora of metabolites including QS molecules and antibiotics

may have evolved as a crucial factor for proper development of immune system. Finally, the beneficial effects of subinhibitory antibiotics such as reduced mortality and morbidity, reduced subclinical disease, improved health and better efficiency of feed conversion in animals may be also viewed from the antibiotic signalling point of view. One of the possible explanations that has been proposed is supression of subclinical infections. The concentrations of antibiotics used, however, are not in the lethal range for bacteria and it is unlikely that pathogens, if any, are killed by low concentrations of antibiotics. It is likely that the effect of subinhibitory growth-promoting antibiotics is complex and involves pleiotropic regulation of the physiology and metabolic state of an entire microbiota as well as of the regulatory mechanisms operating in the host animal. The mechanisms of subinhibitory antibiotics may be through the modulation of the QS networks operating within the gut bacterial community resulting in suppression of virulence factors, redistribution of metabolic fluxes for better feed conversion and other regulatory effects. In the host, subinhibitory antibiotics may modulate the immunity and, through the anti-inflammatory activities, suppress subclinical chronic inflammation draining the host resources. Since this effect is pleotropic, it is probably difficult to reproduce it through the use of pre and/or probiotics. Other multifunctional ligands mimicking these properties of antibiotics may be tried as the alternatives for subinhibitory antibiotics.

A huge number of signalling and regulatory networks operate at every level of organization of living matter, from the cell to ecosystems. We are only beginning to reveal the role played by antibiotics in these processes. Another aspect of antibiotic role in natural ecosystems is the involvement in processes happening at the genetic level. While phenotypic responses are essentially reversible, changes at the genotype level, once they successfully passed through the sieve of natural selection, may become fixed in populations and may even rapidly increase in frequencies if they confer a sufficient selective advantage. In this regard, the effect of antibiotics on genetic processes may have a long-term impact, which is difficult, if not possible, to reverse.

Genotypic responses to antibiotic signalling

Unlike the situation with physiological responses, the effect of low-dose antibiotics, if any, is almost uniform and results in enhanced antibiotic resistance gene transfer, often conferring resistance to structurally unrelated antibiotics. It was found, for example, that subinhibitory concentrations of β-lactam antibiotics increase the transfer of tetracycline resistance plasmids in *S. aureus* by up to 1000-fold (Barr *et al*., 1986). Pre-growth of a donor *Bacteroides* strain on a low concentration of tetracycline also appears to accelerate the mobilization of a resident non-conjugative plasmid by chromosomally encoded tetracycline conjugal elements (Valentine *et al*., 1988). The exposure of donor *Bacteroides* cells to low concentration of tetracycline appears to be a prerequisite for the excision of the CTnDOT family of conjugative transposons from the chromosome and conjugal transfer of the excised elements; virtually no gene transfer occurs without tetracycline induction of donor cells (Stevens *et al*., 1993; Whittle *et al*., 2002). Incorporation of tetracycline at subinhibitory concentrations in the mating medium also substantially enhances Tn*916*-mediated conjugal transfer (Showsh and Andrews, 1992). The similar stimulatory effect of tetracycline on conjugation transfer was also demonstrated for the conjugative transposon Tn*925* (Torres *et al*., 1991). These *in vitro* results have also been reproduced using *in vivo* models. In gnotobiotic mice, for example, the presence of a low concentration of tetracycline in drinking water increased the frequency of transfer of conjugative transposon Tn*1545* from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts by about 10-fold (Doucet-Populaire *et al*., 1991). In gnotobiotic rats, selection for the resistant phenotype was the major factor causing higher numbers of Tn*916* transconjugants in the presence of tetracycline (Bahl *et al*., 2004). Therefore, the enhancement of conjugal transfer of antibiotic resistance-carrying transposons in the presence of subinhibitory concentration of antibiotics is not only an *in vitro* laboratory phenomenon but also takes place in gut ecosystems of animal models. In the environment as well, exposure to a low-dose antibiotic may lead to the mobile element-mediated dissemination of antibiotic resistance genes (Knapp *et al*., 2008).

Among the best-studied mechanisms involved in the enhancement of gene transfer are those mediated through the translational attenuation by tetracycline (Moon *et al*., 2005). According to this model, this attenuation results in increased production of RteA and RteB, leading to activation of *rteC* transcription. In turn, RteC activates transcription of excision genes of CTnDOT and other mobile elements (Moon *et al*., 2005). At subinhibitory tetracycline concentration, the excision is not associated with growth phase (Song *et al*., 2009). Another, less specific mechanism of gene transfer enhancement operates through the structurally unrelated but the SOS response-inducing antibiotics. In particular, DNA-damaging agents such as mitomycin C and antibiotics such as fluoroquinolones and dihydrofolate reductase inhibitors may increase the rate of horizontal gene transfer more than 300-fold (Beaber *et al*., 2004). More importantly, the use of the SOS response-inducing antibiotics may co-select other antibiotic resistance genes that are physically linked in a mobile genetic element (Hastings *et al*., 2004). Although it was initially assumed that the

SOS response is triggered exclusively by DNA-damaging agents, other classes of antibiotics, not interfering with DNA metabolism, may also induce the genuine SOS response. This was demonstrated for the action of β-lactam antibiotics on *S. aureus* (Miller *et al*., 2004). The consequences of this response were elevated rates of horizontal transfer of the staphylococcal virulence genes (Ubeda *et al*., 2005; Maiques *et al*., 2006). In broader terms, control of horizontal transfer of integrative and conjugative elements, which are found in many bacterial genomes and which encode a variety of properties besides the antibiotic resistance and virulence, may also be regulated through the SOS response and environmental signals (Auchtung *et al*., 2005; Bose *et al*., 2008).

The general conclusion from the publications discussed above is that antibiotics may have a signalling function stimulating horizontal gene exchange in microbial ecosystems. This signalling function is unlikely to be limited exclusively to the enhancement of gene transfer processes but may also have a wider implication affecting the genetic variability of microbiota in general. For instance, exposure of *Mycobacterium fortuitum* to subinhibitory concentrations of fluoroquinolone significantly increases mutation rates (Gillespie *et al*., 2005). The components of the SOS response, UmuD(2) and RecA, may regulate the mutagenic activity of the error-prone DNA polymerase DinB (Godoy *et al*., 2007). It is not clear, however, whether the exposure to subinhibitory antibiotic concentrations does indeed introduce DNA damage to elicit the complete SOS response, or it results in ovexpression of DinB, which contributes to the enhanced mutagenesis in the absence of DNA damage (Kim *et al*., 1997). In addition to the SOS response, there are probably a number of other mechanisms by which the subinhibitory antibiotics may increase mutation rates. In pneumococci, exposure to quinolones, aminoglycosides and penicillin at subinhibitory concentrations may result in increased mutability, without the apparent involvement of SOS-response components (Henderson-Begg *et al*., 2006; Cortes *et al*., 2008).

It is not entirely clear why this mechanism of accelerated genetic variability and gene exchange by low-dose antibiotics has been selected in the course of evolution. One of the explanations may be that this is a mechanism for exploring new niches in an existing ecosystem or expanding into an entirely new ecosystem when an ecosystem operates close to the limits of the carrying capacity. Probably this mutation-and-gene exchange acceleration mechanism has been one of the major driving forces shaping very complex microbial ecosystems, with almost every possible niche employed, as we know them today. This mechanism probably also contributes to the ability of microbiota to probe the environment in the search for new opportunities to expand. One of such examples is *P. aeruginosa*, which may be encountered in many natural ecosystems as well as in clinical settings (Khan *et al*., 2007). Genetic adaptation of *P. aeruginosa* to the niche in the airways of cystic fibrosis patients includes numerous genome-wide mutations thus making it systematically different from the wild-type bacterium (Smith *et al*., 2006; Mena *et al*., 2008) and resulting in specific changes of metabolism (D'Argenio *et al*., 2007). Since the chronic infection is very difficult, if not possible, to eradicate, maintenance antibiotic therapy is required to slow the decline in pulmonary function, which may be one of the factors contributing to the appearance of the genome-wide adaptive mutations in *P. aeruginosa*.

The extent and consequences of human intervention with the large-scale production and release of antibiotic substances that normally operate at low concentrations in natural ecosystems are largely unknown. The only consequence we know definitely is the dramatic increase in frequencies of antibiotic resistance gene carriage by pathogenic and commensal microbiota. Can this trend be reversed? Although it is generally thought that due to the fitness cost of antibiotic resistance will be subjected to negative selection once the antibiotic selective pressure is removed, there are many examples demonstrating how the remarkable plasticity of bacteria allows them to ameliorate this cost (Lenski, 1997; Enne *et al*., 2005; Ramadhan and Hegedus, 2005; Andersson, 2006; Nilsson *et al*., 2006). Moreover, the acquisition of the antibiotic resistance genotype may actually increase the fitness of certain bacteria in the absence of antibiotic selective pressure thus allowing the rapid emergence and dissemination on a worldwide scale (Enne *et al*., 2004; Luo *et al*., 2005). It is not known to what extent the level of antibiotic resistance in natural ecosystems has been affected by the human use of antibiotics. If to the same level as it is seen in pathogens and commensals, then the antibiotic-mediated cross-talk in natural ecosystems may be seriously compromised, especially if the mechanisms of resistance include modification or degradation of antibiotic ligands.

Antibiotics and resistance to them: selection of combinations

In this review, resistance to antibiotics has been discussed mainly from an ecological point of view as 'natural' mechanisms of regulating antibiotic cross-talk. In some groups of enzymes conferring antibiotic resistance, it is still possible to track structure-and-function relationships that eventually lead to the development of antibiotic resistance properties. For instance, the therapeutic action of β-lactams is achieved through the binding to PBPs thus disrupting the growth and structural integrity of bacterial cell walls. Structurally, β-lactamases are similar to PBPs

that play an important role in bacterial cell cycle (Macheboeuf *et al*., 2006). The transpeptidation reaction performed by PBPs serves to stabilize the cell wall by cross-linking the glycan strands during peptidoglycan synthesis. The structure modifications during the evolution of the intermediate antibiotic resistance enzyme were apparently through the loss of interaction with the peptidoglycan moieties (Meroueh *et al*., 2003). Given the extended evolutionary history and diversity, wide distribution in the environment and efficient catalytic properties of β-lactamases, they have probably served as 'moderators' in β-lactam cross-talk for a long time. It is not surprising therefore that these genes were rapidly picked up from the environmental genetic reservoirs and disseminated into commensal and pathogenic microbiota following the introduction of β-lactam antibiotics into clinical, veterinary and other practices.

It is unlikely that antibiotic resistance genes and enzymes, as we know them today, all have evolved from the antibiotic cross-talk mechanism. This, apparently, cannot be the case with synthetic antibiotics that have no natural analogues such as quinolones. The bactericidal activity of quinolones is mediated through the formation of DNA gyrase–quinolone–DNA ternary complex thus arresting replication fork progression and leading to the cell death (Hiasa and Shea, 2000). The acquired resistance to quinolones is due to the *qnr* gene, which encodes a 218 aa protein belonging to the pentapeptide repeat family, with sequence homology with the immunity protein McbG (Tran and Jacoby, 2002). The protection mechanism operates through the binding of Qnr to DNA gyrase in the early stages of gyrase–DNA complex formation and, by lowering gyrase binding to DNA, Qnr may reduce the amount of holoenzyme-DNA targets for quinolone inhibition (Tran *et al*., 2005). The genes encoding the pentapeptide repeat family proteins are widespread in nature and cloning and phylogenetic reconstruction suggested that the *qnr* genes have been acquired from the environmental bacteria (Poirel *et al*., 2005; Sánchez *et al*., 2008). The three-dimensional structure of the MfpA protein from *Mycobacterium tuberculosis* is remarkably similar to DNA in terms of size, shape and electrostatic properties and these properties may explain its inhibitory effect on DNA gyrase and quinolone resistance (Hegde *et al*., 2005). It was suggested that the original function of MfpA is in providing DNA topological assistance when needed, but maintaining a condensed chromosome and preventing undesired topological changes during periods of replicative senescence (Hegde *et al*., 2005). Thus the enzyme possibly involved in DNA metabolism in environmental bacteria appeared to be also protective against a synthetic antibiotic and, once a strong selective pressure has been applied, the corresponding gene was picked up from the environmental pool and rapidly penetrated into pathogenic microbiota (Aminov and Mackie, 2007). It still can be argued that due to the enormous metabolic diversity of environmental microbiota, the structurally similar compounds can be found in nature. Pseudomonads and other bacteria produce a quorum-signalling molecule, 2-heptyl-3-hydroxy-4-quinolone, which belongs to the family of 2-alkyl-4-quinolones (Dubern and Diggle, 2008). These molecules have been initially described for their antibiotic activities (Hays *et al*., 1945). Another *P. aeruginosa* QS molecule, *N*-(3-oxododecanoyl) homoserine lactone, and its non-enzymatically formed product, 3-(1-hydroxydecylidene)-5-(2-hydroxyethyl)pyrrolidine-2, 4-dione, both display potent antibacterial activities (Kaufmann *et al*., 2005). These observations suggest that the functions of the known QS molecules and antibiotics overlap thus further supporting the hypothesis about the concentration-dependent effects of small molecules and the possible function of antibiotics as signal mediators.

The acquisition of metabolic genes by bacteria from their environment to provide an unrelated function, such as antibiotic resistance, is not limited to protection against synthetic antibiotics. Even in the case of natural antibiotics in the tetracycine family, the mechanisms of resistance may include ion pumps that are involved in a number of other cellular functions such as Na+ and K+ exchange for protons (Krulwich *et al*., 2001) or the expression of genes involved in biosynthesis of extracellular lipopolysaccharides and capsular polysaccharides (Kazimierczak *et al*., 2009). The horizontal gene transfer step may be not necessary if the cellular metabolic mechanisms affected have a sufficiently broad specificity to cope with novel substrates such as antibiotics. For example, the natural functions of bacterial multi-drug efflux pumps, which are usually chromosomally encoded and are implicated in antibiotic resistance, may include detoxification, virulence, cell homeostasis and intercellular signal trafficking (Piddock, 2006; Martínez *et al*., 2009). These observations can be integrated within the concept of co-optation in which the enormous metabolic diversity of microbiota may provide the concomitant functions such as resistance to antibiotics.

Practical implications: prediction of antibiotic resistance

Initially, experimental approaches to predict the evolution of antibiotic resistance genes have included *in vitro* evolution modelling and screening for cryptic antibiotic resistance genes (Hall, 2004). A broader conceptual framework to predict antibiotic resistance was proposed that included many variables such as the estimates of potentiality and probability affecting the actuality, e.g. the actual antibiotic resistance in bacterial populations (Martínez *et al*., 2007). Genetic mechanisms that may

contribute to evolution of antibiotic resistance were also discussed (Courvalin, 2008). No formalized theorethical framework for prediction of antibiotic resistance, however, presently exists due to the lack of knowledge in several key areas such as the true extent of microbial metabolic diversity that may contribute to novel antibiotic resistance, the rates of mutation and horizontal gene transfer (HGT) in natural ecosystems, and the extent of genetic interaction between different ecosystems. In the face of the growing antibiotic resistance problem, however, even the scenarios of antibiotic resistance development that are based on incomplete knowledge can be of value in an attempt to preserve the efficacy of novel antibiotics. Potentiality, probability and actuality (Martínez *et al*., 2007) for tigecycline resistance will be discussed in this section.

The first- and second-generation tetracylines have gradually lost their efficiency due to the widespread resistance conferred mainly by the ribosomal protection and efflux mechanisms (Chopra and Roberts, 2001; Roberts, 2005). The first representative of the third generation of tetracyclines (collectively called glycylcyclines), tigecycline (the minocycline derivative 9-*tert*-butyl-glycylamido-minocycline, GAR-936), was approved by the US Food and Drug Administration (FDA) in 2005 and in 2006 in the European Union. This antibiotic was shown to be effective against bacterial isolates containing the two major determinants responsible for tetracycline resistance (Petersen *et al*., 1999), clinical isolates of *Acinetobacter* (Henwood *et al*., 2002), non-tuberculous mycobacteria (Wallace *et al*., 2002), enterococci (Mercier *et al*., 2002; Lefort *et al*., 2003; Nannini *et al*., 2003), *S. aureus* (Mercier *et al*., 2002; Petersen *et al*., 2002), *Legionella pneumophila* (Edelstein *et al*., 2003), *Stenotrophomonas maltophilia* (Betriu *et al*., 2002), and other clinical isolates (Betriu *et al*., 2002; Abbanat *et al*., 2003; Milatovic *et al*., 2003). Thus it is a valuable therapeutic option when dealing with hard-to-treat multi-drug-resistant infections such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), penicillin-resistant *Streptococcus pneumoniae* and the β-lactamase-producing *Enterobacteriaceae*. It can be concluded that the penetration of tigecycline resistance into pathogenic microbiota is very low at this time.

Still, there are examples of resistance to tigecycline that is conferred by two mechanisms, efflux from the cell and drug modification. In *P. aeruginosa*, the mechanism of decreased susceptibility to tigecycline is through the resistance nodulation division (RND) family of efflux pumps, in particular, the MexXY–OprM complex (Dean *et al*., 2003). However, the compensatory mechanisms of *P. aeruginosa* against tigecycline in the absence of MexXY–OprM included two other efflux pumps, MexAB–OprM and MexCD–OprJ, which, in norm, are 'specialized' in the efflux of second-generation semisynthetic tetracyclines, doxycycline and minocycline. In *Proteus mirabilis*, reduced susceptibility to tigecycline is also mediated by another representative of the RND family of efflux pumps, encoded by the *acrAB* homologue of *E. coli*, the AcrAB–TolC system (Visalli *et al*., 2003; Ruzin *et al*., 2005). In clinical isolates of *Acinetobacter baumannii* the reduced susceptibility to tigecycline was also attributed to an elevated expression of the RND family of efflux pumps, namely the AdeABC and AdeIJK systems (Peleg *et al*., 2007; Damier-Piolle *et al*., 2008). Elevated expression of another efflux pump in the RND family, AcrAB, was implicated in reduced tigecycline susceptibility in *Enterobacter cloacae* and *E. coli* (Keeney *et al*., 2007; 2008). Thus, this *post factum* determined potentiality involves non-specific efflux mechanisms, which are overexpressed most probably due to mutations in the regulatory system (Peleg *et al*., 2007). Because of the chromosomal localization and the involvement of several genes, the probability of immediate dissemination of this mechanism of resistance is low.

Another mechanism of glycylcycline resistance, through chemical transformation, is encoded by *tet*(X). The gene was detected in anaerobic *Bacteroides* species more than two decades ago (Guiney *et al*., 1984). Paradoxically, in the original host it is cryptic and does not confer resistance to tetracyclines because the encoded enzyme is a flavin-dependent monooxygenase that requires molecular oxygen in order to transform the tetracycline substrates (Yang *et al*., 2004). It appears that under aerobic condition and aerobic host this enzyme has a broad specificity and can degrade virtually all tetracyclines including tigecycline (Yang *et al*., 2004; Moore *et al*., 2005).

To access the evolutionary origin of *tet*(X), the similarity search was performed against databases, which produced the highest scoring matches with the TetX proteins as well as with the flavoprotein monooxygenases (data now shown), a diverse group of enzymes that catalyse region-selective hydroxylation of organic substrates and which are found in many metabolic pathways in all three domains of life (Harayama *et al*., 1992). These enzymes are divided into six classes, from A to F, based on sequence and three-dimensional structural data (van Berkel *et al*., 2006). The class A monooxygenases, to which TetX belongs, are usually involved in the microbial degradation of aromatic compounds by *ortho*- or *para*-hydroxylation of the aromatic ring (Moonen *et al*., 2002). Phylogenetic analyses demonstrated that these enzymes display quite extraordinary diversity and are highly incongruent with the 16S rRNA gene-based trees suggesting extensive horizontal gene transfer events in the evolution of this class of flavoprotein monooxygenases (data not shown). Another prominent feature of these genes is that duplication played a substantial role in their evolution. The ecology of bacteria that harbour these genes encom-

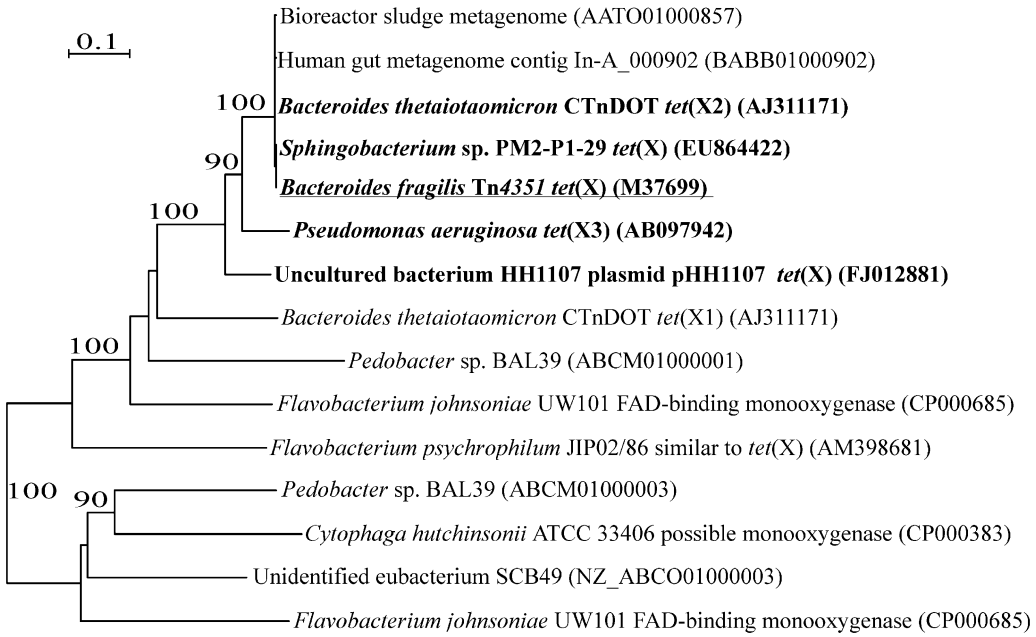

Fig. 1. A neighbour-joining tree of *tet*(X) and flavoprotein monooxygenase-encoding genes. Numbers above each node show the percentage of tree configurations that occurred during 1000 bootstrap trials. The scale bar is in fixed nucleotide substitutions per sequence position. GenBank accession numbers of nucleotide sequences used in this analysis are given in parenthesis. The cases of confirmed functionality of *tet*(X) in *E. coli* or in original hosts (resistance to the first- and second-generation tetracyclines) are shown in bold and the case of heterologous tigecycline resistance expression in *E. coli* is underlined.

passes various environmental niches, including soil, water and intestinal ecosystems, while some of them are known to be opportunistic pathogens. The range of biochemical reactions performed by this class of enzymes is quite broad and, interestingly, includes modification of other than tetracycline aromatic polyketides such as rifampin (Andersen *et al*., 1997), mithramycin (Prado *et al*., 1999), griseorhodin (Li and Piel, 2002), chromomycin (Menendez *et al*., 2004) and auricin (Novakova *et al*., 2005).

For more accurate and sensitive analysis of the evolutionary branch that has led to the appearance of *tet*(X), sequence databases were searched with the protein translation of the original *tet*(X) gene query (GenBank Accession No. M37699 against translated nucleotide databases including environmental DNA and the high-scoring (*E*-values < $5e^{-53}$) hits were used in phylogenetic reconstruction using the corresponding nucleotide sequences (Fig. 1). The sequences with the highest similarity to *tet*(X) were found predominantly in bacteria belonging to the *Bacteroidetes* phylum (Fig. 1). This analysis confirmed the gene duplication events in some bacterial genomes (e.g. *Flavobacterium johnsoniae* UW101 or *Pedobacter* sp. BAL39). The ancestor clade leading to the emergence of *tet*(X) was localized in the genome sequence of the fish pathogen *Flavobacterium psychrophilum* (Duchaud *et al*., 2007; Fig. 1). The highly similar (≥ 99%) *tet*(X) nucleotide sequences form a tight cluster including the sequences from intestinal *Bacteroides* species (Speer *et al*., 1991; Whittle *et al*., 2001), environmental *Sphingobacterium* sp. (Ghosh *et al*., 2009), human gut metagenome (Kurokawa *et al*., 2007), and the metagenome of biological phosphorus removal sludge community (García Martín *et al*., 2006) (Fig. 1). The functional tigecycline resistance is experimentally confirmed only for *tet*(X) from Tn*4351* (Moore *et al*., 2005) but, because of the virtual identity of sequences in the cluster, all of them must confer resistance to tigecycline. More distant sequences are from an opportunistic pathogen *P. aeruginosa* (AB097942), which is resistant to minocycline, and from an environmental plasmid (FJ012881), which expresses tetracycline resistance in *E. coli*. Thus, *tet*(X) can be encountered in several ecosystems including the human gut, clinical settings, soil and a sludge bioreactor. We have also detected its presence in the pig intestinal microbiota using *tet*(X)-specific primers (data not shown). It is not clear what kind of selection has contributed to the widespread dissemination of *tet*(X). In anaerobic *Bacteroides*, where the gene is cryptic, this was probably a result of co-selection by aminoglycosides and macrolides due to the genetic linkage with *addS* and *ermF* in a mobile element (Whittle *et al*., 2001), while in aerobic bacteria the additional selective forces involved could include direct selection by older-generation tetracyclines.

The genetic context of the *tet*(X) orthologue in the genome of *F. psychrophilum* JIP02/86 is associated with a

putative 28.5 kb mobile element (AM398681, analysis not shown). Of particular interest is the linkage of the TetX and XerD ORFs with the homologue of the regulatory protein RteC, which is essential for self-transfer of conjugative transposons and mobilization of co-resident plasmids in *Bacteroides* species and which is regulated by tetracycline (Stevens *et al*., 1993). In the genome of *F. johnsoniae* UW101, RteC is located within a putative transposon, whose ORFs share homology with *Bacteroides* conjugative transposons (CP000685). The first heterologously functional but cryptic in the original host *tet*(X) genes were found to be located on large *Bacteroides* plasmids, pBF4 and pCP1, which can be transferred to *E. coli* by conjugation and express the resistance phenotype in this host (Guiney *et al*., 1984). Further investigations established that these genes are also the part of *Bacteroides* conjugative transposons, Tn*4351* and Tn*4400* (Robillard *et al*., 1985; Shoemaker *et al*., 1985). The mobility of conjugative transposon Tn*4351* was confirmed for at least among the bacterial genera in the *Bacteroidetes* phylum (McBride and Baker, 1996). TetX1- and TetX2-encoding sequences were initially identified on a CTnDOT transposon from a human gut commensal bacterium *Bacteroides* thetaiotaomicron but presumably this transposon and its derivatives, with the *ermF* region that contain these two tetracycline resistance genes, may present in many other intestinal *Bacteroides* species as well (Whittle *et al*., 2001). The environmental *Sphingobacterium* sp. isolate carries *tet*(X) on a transposon-like element, Tn*6031*, which is very similar to CTnDOT (Ghosh *et al*., 2009). Our analysis of the limited regions of sequence data available that surround *tet*(X) in the human gut metagenome (BABB01000902) also demonstrated a synteny with CTnDOT (data not shown). And finally, the gene was discovered on a large 58 kb plasmid, which was transferred from soil microbiota to *E. coli* recipient by conjugation (Heuer *et al*., 2008). The *Acinetobacter* spp. are probably putative hosts for these low G+C plasmids in soil ecosystem, which is of concern because the closely related multi-drug-resistant *A. baumannii* is one of the currently emerging threats in hospitals (Dijkshoorn *et al*., 2007). Tigecycline is currently proposed as a new treatment choice against *A. baumannii* (Bosó-Ribelles *et al*., 2008) but this bacterium already poses a significant problem with the easily emerging resistance during tigecycline therapy, albeit conferred by a mechanism other than TetX (Peleg *et al*., 2007; Damier-Piolle *et al*., 2008). Thus, the horizontal gene transfer potentials of *tet*(X) are much higher than that of the RND-mediated resistance making the former a more likely candidate for the possible emergence of tigecycline resistance among pathogens.

Unlike the indiscriminate use of earlier tetracyclines, the current use of tigecycline is restricted and, based on successful results of large clinical trials, it is prescribed mostly for the treatment of complicated skin and skin structure infections and intra-abdominal infections in humans as intravenous infusions (Babinchak *et al*., 2005; Ellis-Grosse *et al*., 2005). During the tigecycline treatment of healthy volunteers, the serum concentration of the drug was less than 1 $\mu g\ ml^{-1}$ but intestinal concentrations were much higher, on average 6 μg per gram of faeces on day 8, which is consistent with the biliary elimination of the drug (Nord *et al*., 2006). The tigecycline-resistant bacteria selected in the human gastrointestinal system during this treatment included two *Klebsiella pneumoniae* and five *E. cloacae* strains (Nord *et al*., 2006), with the mechanism(s) of resistance requiring further investigation.

In summary: (i) originating from the widespread flavoprotein monooxygenase family, TetX possesses efficient tigecycline inactivation capability, (ii) the gene encoding TetX is located on multiple mobile genetic elements, (iii) the gene was possibly pre-selected by the older tetracyclines and can be detected in several ecosystems including the human gut, and (iv) strong selective pressure is expected in the gut of patients undergoing tigecycline therapy. These prerequisites make TetX as a most likely mechanism and the human gut as a most likely ecosystem for the emergence of tigecycline-resistant pathogens. The limiting factor in *tet*(X) dissemination is an anaerobic metabolism of potential bacterial hosts, which is unable to support its expression and therefore the gene cannot be selected once the antibiotic pressure is applied.

Concluding remarks

A broader overview of the role of antibiotics and antibiotic resistance in different environments has changed current paradigms. Armed with this knowledge, an attempt was made to model a scenario based on information we have about resistance to a novel antibiotic. Future developments will determine if present-day knowledge is sufficient to predict resistance to novel antibiotics. The areas that merit further investigations include the evolutionary, environmental and regulatory aspects of antibiotics and antibiotic resistance. We still have very little idea about the impact of the large-scale antibiotic synthesis and release on natural microbiota. Increased frequencies of antibiotic resistance gene carriage by pathogens and some commensal bacteria are well documented but what about other ecosystems? While good progress has been made in identification of environmental reservoirs of antibiotic resistance, almost nothing is known about the environmental concentrations of antibiotics. Regulatory and signalling aspects that include identification of molecular targets of subinhibitory antibiotics in all three domains of life and how they interact with other regulatory networks remain a priority. In bacteria, due to the complexity of regulatory networks integrating multiple environmental

signals and the dynamic nature of responses, the experimental variables must be standardized in order for the results to be comparable. Among the eukaryotic organisms, some progress has been made in target identification but still the signalling mechanisms of antibiotics that are beyond their intended antibacterial use remain largely unknown. The availability of the large number of antibiotics, already approved for human use, makes them a valuable source for treatment of other, than infectious, diseases in humans and animals. Almost nothing is known about the effects of subinhibitory antibiotics on archaea despite that they are prominent members in many microbial communities. Research in these areas will not only help to understand more about antibiotics and antibiotic resistance in natural ecosystems but also will aid in making responsible choices when interacting with these ecosystems in the course of clinical or agricultural use of antibiotics.

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References

Abbanat, D., Macielag, M., and Bush, K. (2003) Novel antibacterial agents for the treatment of serious Gram-positive infections. *Expert Opin Invest Drugs* **12:** 379–399.

Ahmed, N.A., Petersen, F.C., and Scheie, A.A. (2007) AI-2 quorum sensing affects antibiotic susceptibility in *Streptococcus anginosus*. *J Antimicrob Chemother* **60:** 49–53.

Allen, H.K., Moe, L.A., Rodbumrer, J., Gaarder, A., and Handelsman, J. (2009) Functional metagenomics reveals diverse β-lactamases in a remote Alaskan soil. *ISME J* **3:** 243–251.

Aminov, R.I., and Mackie, R.I. (2007) Evolution and ecology of antibiotic resistance genes. *FEMS Microbiol Lett* **271:** 147–161.

Andersen, S.J., Quan, S., Gowan, B., and Dabbs, E.R. (1997) Monooxygenase-like sequence of a *Rhodococcus equi* gene conferring increased resistance to rifampin by inactivating this antibiotic. *Antimicrob Agents Chemother* **41:** 218–221.

Andersson, D.I. (2006) The biological cost of mutational antibiotic resistance: any practical conclusions? *Curr Opin Microbiol* **9:** 461–465.

Auchtung, J.M., Lee, C.A., Monson, R.E., Lehman, A.P., and Grossman, A.D. (2005) Regulation of a *Bacillus subtilis* mobile genetic element by intercellular signaling and the global DNA damage response. *Proc Natl Acad Sci USA* **102:** 12554–12559.

Babinchak, T., Ellis-Grosse, E., Dartois, N., Rose, G.M., and Loh, E. (2005) The efficacy and safety of tigecycline for the treatment of complicated intraabdominal infections: analysis of pooled clinical trial data. *Clin Infect Dis* **41:** S354–S367.

Bagge, N., Schuster, M., Hentzer, M., Ciofu, O., Givskov, M., Greenberg, E.P., and Høiby, N. (2004) *Pseudomonas aeruginosa* biofilms exposed to imipenem exhibit changes in global gene expression and beta-lactamase and alginate production. *Antimicrob Agents Chemother* **48:** 1175–1187.

Bahl, M.I., Sorensen, S.J., Hansen, L.H., and Licht, T.R. (2004) Effect of tetracycline on transfer and establishment of the tetracycline-inducible conjugative transposon Tn*916* in the guts of gnotobiotic rats. *Appl Environ Microbiol* **70:** 758–764.

Bainton, N.J., Stead, P., Chhabra, S.R., Bycroft, B.W., Salmond, G.P., Stewart, G.S., and Williams, P. (1992) *N*-(3-oxohexanoyl)-L-homoserine lactone regulates carbapenem antibiotic production in *Erwinia carotovora*. *Biochem J* **288:** 997–1004.

Baquero, F., Martínez, J.L., and Cantón, R. (2008) Antibiotics and antibiotic resistance in water environments. *Curr Opin Biotechnol* **19:** 260–265.

Barr, V., Barr, K., Millar, M.R., and Lacey, R.W. (1986) Beta-lactam antibiotics increase the frequency of plasmid transfer in *Staphylococcus aureus*. *J Antimicrob Chemother* **17:** 409–413.

Beaber, J.W., Hochhut, B., and Waldor, M.K. (2004) SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* **427:** 72–74.

van Berkel, W.J., Kamerbeek, N.M., and Fraaije, M.W. (2006) Flavoprotein monooxygenases, a diverse class of oxidative biocatalysts. *J Biotechnol* **124:** 670–689.

Betriu, C., Rodriguez-Avial, I., Sánchez, B.A., Gómez, M., and Picazo, J.J. (2002) Comparative *in vitro* activities of tigecycline (GAR-936) and other antimicrobial agents against *Stenotrophomonas maltophilia*. *J Antimicrob Chemother* **50:** 758–759.

Blickwede, M., Goethe, R., Wolz, C., Valentin-Weigand, P., and Schwarz, S. (2005a) Molecular basis of florfenicol-induced increase in adherence of *Staphylococcus aureus* strain Newman. *J Antimicrob Chemother* **56:** 315–323.

Blickwede, M., Wolz, C., Valentin-Weigand, P., and Schwarz, S. (2005b) Influence of clindamycin on the stability of *coa* and *fnbB* transcripts and adherence properties of *Staphylococcus aureus* Newman. *FEMS Microbiol Lett* **252:** 73–78.

Boisset, S., Geissmann, T., Huntzinger, E., Fechter, P., Bendridi, N., Possedko, M., *et al*. (2007) *Staphylococcus aureus* RNAIII coordinately represses the synthesis of virulence factors and the transcription regulator Rot by an antisense mechanism. *Genes Dev* **21:** 1353–1366.

Bose, B., Auchtung, J.M., Lee, C.A., and Grossman, A.D. (2008) A conserved anti-repressor controls horizontal gene transfer by proteolysis. *Mol Microbiol* **70:** 570–582.

Bosó-Ribelles, V., Romá-Sánchez, E., Carmena, J., Cáceres, C., and Bautista, D. (2008) Tigecycline: a new treatment choice against *Acinetobacter baumannii*. *Recent Patents Anti-Infect Drug Disc* **3:** 117–122.

Bronner, S., Monteil, H., and Prévost, G. (2004) Regulation of virulence determinants in *Staphylococcus aureus*: complexity and applications. *FEMS Microbiol Rev* **28:** 183–200.

Bush, K. (1998) Metallo-beta-lactamases: a class apart. *Clin Infect Dis* **27:** S48–S53.

Chee-Sanford, J., Aminov, R.I., Garrigues, N., Krapac, I., and Mackie, R. (2001) Occurrence and diversity of tetracycline resistance genes in lagoons and groundwater underlying two swine production facilities. *Appl Environ Microbiol* **67:** 1494–1502.

Chee-Sanford, J.C., Mackie, R.I., Koike, S., Krapac, I.G., Lin, Y.F., Yannarell, A.C., *et al.* (2009) Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste. *J Environ Qual* **38:** 1086–1108.

Chopra, I., and Roberts, M. (2001) Tetracycline antibiotics: mode of action, applications, molecular biology, and epidemiology of bacterial resistance. *Microbiol Mol Biol Rev* **65:** 232–260.

Cortes, P.R., Piñas, G.E., Albarracin Orio, A.G., and Echenique, J.R. (2008) Subinhibitory concentrations of penicillin increase the mutation rate to optochin resistance in *Streptococcus pneumoniae*. *J Antimicrob Chemother* **62:** 973–977.

Coulthurst, S.J., Barnard, A.M., and Salmond, G.P. (2005) Regulation and biosynthesis of carbapenem antibiotics in bacteria. *Nat Rev Microbiol* **3:** 295–306.

Courvalin, P. (2008) Predictable and unpredictable evolution of antibiotic resistance. *J Intern Med* **264:** 4–16.

Cox, A.R., Thomson, N.R., Bycroft, B., Stewart, G.S., Williams, P., and Salmond, G.P. (1998) A pheromone-independent CarR protein controls carbapenem antibiotic synthesis in the opportunistic human pathogen *Serratia marcescens*. *Microbiology* **144:** 201–209.

D'Argenio, D.A., Wu, M., Hoffman, L.R., Kulasekara, H.D., Déziel, E., Smith, E.E., *et al.* (2007) Growth phenotypes of *Pseudomonas aeruginosa lasR* mutants adapted to the airways of cystic fibrosis patients. *Mol Microbiol* **64:** 512–533.

D'Costa, V.M., McGrann, K.M., Hughes, D.W., and Wright, G.D. (2006) Sampling the antibiotic resistome. *Science* **311:** 374–377.

D'Costa, V.M., Griffiths, E., and Wright, G.D. (2007) Expanding the soil antibiotic resistome: exploring environmental diversity. *Curr Opin Microbiol* **10:** 481–489.

Damier-Piolle, L., Magnet, S., Brémont, S., Lambert, T., and Courvalin, P. (2008) AdeIJK, a resistance-nodulation-cell division pump effluxing multiple antibiotics in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* **52:** 557–562.

Dantas, G., Sommer, M.O., Oluwasegun, R.D., and Church, G.M. (2008) Bacteria subsisting on antibiotics. *Science* **320:** 100–103.

Davies, J., Spiegelman, G.B., and Yim, G. (2006) The world of subinhibitory antibiotic concentrations. *Curr Opin Microbiol* **9:** 445–453.

Dean, C.R., Visalli, M.A., Projan, S.J., Sum, P.E., and Bradford, P.A. (2003) Efflux-mediated resistance to tigecycline (GAR-936) in *Pseudomonas aeruginosa* PAO1. *Antimicrob Agents Chemother* **47:** 972–978.

Del Rosso, J.Q. (2007) Recently approved systemic therapies for acne vulgaris and rosacea. *Cutis* **80:** 113–120.

Derzelle, S., Duchaud, E., Kunst, F., Danchin, A., and Bertin, P. (2002) Identification, characterization, and regulation of a cluster of genes involved in carbapenem biosynthesis in *Photorhabdus luminescens*. *Appl Environ Microbiol* **68:** 3780–3789.

Dijkshoorn, L., Nemec, A., and Seifert, H. (2007) An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. *Nat Rev Microbiol* **5:** 939–951.

Dong, Y.H., Wang, L.H., Xu, J.L., Zhang, H.B., Zhang, X.F., and Zhang, L.H. (2001) Quenching quorum-sensing-dependent bacterial infection by an *N*-acyl homoserine lactonase. *Nature* **411:** 813–817.

Doucet-Populaire, F., Trieu-Cuot, P., Dosbaa, I., Andremont, A., and Courvalin, P. (1991) Inducible transfer of conjugative transposon Tn*1545* from *Enterococcus faecalis* to *Listeria monocytogenes* in the digestive tracts of gnotobiotic mice. *Antimicrob Agents Chemother* **35:** 185–187.

Dubern, J.F., and Diggle, S.P. (2008) Quorum sensing by 2-alkyl-4-quinolones in *Pseudomonas aeruginosa* and other bacterial species. *Mol Biosyst* **4:** 882–888.

Duchaud, E., Boussaha, M., Loux, V., Bernardet, J.F., Michel, C., Kerouault, B., *et al.* (2007) Complete genome sequence of the fish pathogen *Flavobacterium psychrophilum*. *Nat Biotechnol* **25:** 763–769.

Edelstein, P.H., Weiss, W.J., and Edelstein, M.A. (2003) Activities of tigecycline (GAR-936) against *Legionella pneumophila in vitro* and in guinea pigs with *L. pneumophila* pneumonia. *Antimicrob Agents Chemother* **47:** 533–540.

Ellis-Grosse, E.J., Babinchak, T., Dartois, N., Rose, G., and Loh, E. (2005) The efficacy and safety of tigecycline in the treatment of skin and skin-structure infections: results of 2 double-blind phase 3 comparison studies with vancomycin-aztreonam. *Clin Infect Dis* **41:** S341–S353.

Encinar, J.A., Mallo, G.V., Mizyrycki, C., Giono, L., Gonzalez-Ros, J.M., Rico, M., *et al.* (2001) Human p8 is a HMG-I/Y-like protein with DNA binding activity enhanced by phosphorylation. *J Biol Chem* **276:** 2742–2751.

Enne, V.I., Bennett, P.M., Livermore, D.M., and Hall, L.M. (2004) Enhancement of host fitness by the sul2-coding plasmid p9123 in the absence of selective pressure. *J Antimicrob Chemother* **53:** 958–963.

Enne, V.I., Delsol, A.A., Davis, G.R., Hayward, S.L., Roe, J.M., and Bennett, P.M. (2005) Assessment of the fitness impacts on *Escherichia coli* of acquisition of antibiotic resistance genes encoded by different types of genetic element. *J Antimicrob Chemother* **56:** 544–551.

Fajardo, A., and Martínez, J.L. (2008) Antibiotics as signals that trigger specific bacterial responses. *Curr Opin Microbiol* **11:** 161–167.

Fevre, C., Jbel, M., Passet, V., Weill, F.X., Grimont, P.A., and Brisse, S. (2005) Six groups of the OXY β-lactamase evolved over millions of years in *Klebsiella oxytoca*. *Antimicrob Agents Chemother* **49:** 3453–3462.

Fineran, P.C., Slater, H., Everson, L., Hughes, K., and Salmond, G.P. (2005) Biosynthesis of tripyrrole and beta-lactam secondary metabolites in *Serratia*: integration of quorum sensing with multiple new regulatory components in the control of prodigiosin and carbapenem antibiotic production. *Mol Microbiol* **56:** 1495–1517.

Fuqua, C., Winans, S.C., and Greenberg, E.P. (1996) Census and consensus in bacterial ecosystems: the LuxR–LuxI family of quorum-sensing transcriptional regulators. *Annu Rev Microbiol* **50:** 727–751.

Garau, G., Di Guilmi, A.M., and Hall, B.G. (2005) Structure-

based phylogeny of the metallo-beta-lactamases. *Antimicrob Agents Chemother* **49:** 2778–2784.
García Martín, H., Ivanova, N., Kunin, V., Warnecke, F., Barry, K.W., McHardy, A.C., *et al.* (2006) Metagenomic analysis of two enhanced biological phosphorus removal (EBPR) sludge communities. *Nat Biotechnol* **24:** 1263–1269.
Geiger, T., Goerke, C., Mainiero, M., Kraus, D., and Wolz, C. (2008) The virulence regulator Sae of *Staphylococcus aureus*: promoter activities and response to phagocytosis-related signals. *J Bacteriol* **90:** 3419–3428.
Ghosh, S., Sadowsky, M.J., Roberts, M.C., Gralnick, J.A., and Lapara, T.M. (2009) *Sphingobacterium* sp. strain PM2-P1-29 harbours a functional *tet*(X) gene encoding for the degradation of tetracycline. *J Appl Microbiol* **106:** 1336–1342.
Gillespie, S.H., Basu, S., Dickens, A.L., O'Sullivan, D.M., and McHugh, T.D. (2005) Effect of subinhibitory concentrations of ciprofloxacin on *Mycobacterium fortuitum* mutation rates. *J Antimicrob Chemother* **56:** 344–348.
Girard, G., and Bloemberg, G.V. (2008) Central role of quorum sensing in regulating the production of pathogenicity factors in *Pseudomonas aeruginosa*. *Future Microbiol* **3:** 97–106.
Godoy, V.G., Jarosz, D.F., Simon, S.M., Abyzov, A., Ilyin, V., and Walker, G.C. (2007) UmuD and RecA directly modulate the mutagenic potential of the Y family DNA polymerase DinB. *Mol Cell* **28:** 1058–1070.
Guiney, D.G., Hasegawa, P., and Davis, C.E. (1984) Expression in *E. coli* of cryptic tetracycline resistance genes from *Bacteroides* R plasmids. *Plasmid* **11:** 248–252.
Haas, D., and Défago, G. (2005) Biological control of soil-borne pathogens by fluorescent pseudomonads. *Nat Rev Microbiol* **3:** 307–319.
Hall, B.G. (2004) Predicting the evolution of antibiotic resistance genes. *Nat Rev Microbiol* **2:** 430–435.
Hall, B.G., and Barlow, M. (2004) Evolution of the serine beta-lactamases: past, present and future. *Drug Resist Updat* **7:** 111–123.
Harayama, S., Kok, M., and Neidle, E.L. (1992) Functional and evolutionary relationships among diverse oxygenases. *Annu Rev Microbiol* **46:** 565–601.
Hastings, P.J., Rosenberg, S.M., and Slack, A. (2004) Antibiotic-induced lateral transfer of antibiotic resistance. *Trends Microbiol* **12:** 401–404.
Hays, E.E., Wells, I.C., Katzman, P.A., Cain, C.K., Jacobs, F.A., Thayer, S.A., *et al.* (1945) Antibiotic substances produced by *Pseudomonas aeruginosa*. *J Biol Chem* **159:** 725–750.
Hegde, S.S., Vetting, M.W., Roderick, S.L., Mitchenall, L.A., Maxwell, A., Takiff, H.E., and Blanchard, J.S. (2005) A fluoroquinolone resistance protein from *Mycobacterium tuberculosis* that mimics DNA. *Science* **308:** 1480–1483.
Henderson-Begg, S.K., Livermore, D.M., and Hall, L.M. (2006) Effect of subinhibitory concentrations of antibiotics on mutation frequency in *Streptococcus pneumoniae*. *J Antimicrob Chemother* **57:** 849–854.
Henwood, C.J., Gatward, T., Warner, M., James, D., Stockdale, M.W., Spence, R.P., *et al.* (2002) Antibiotic resistance among clinical isolates of *Acinetobacter* in the UK, and *in vitro* evaluation of tigecycline (GAR-936). *J Antimicrob Chemother* **49:** 479–487.
Hesketh, A., Chen, W.J., Ryding, J., Chang, S., and Bibb, M. (2007) The global role of ppGpp synthesis in morphological differentiation and antibiotic production in *Streptomyces coelicolor* A3(2). *Genome Biol* **8:** R161.
Heuer, H., Kopmann, C., Binh, C.T., Top, E.M., and Smalla, K. (2008) Spreading antibiotic resistance through spread manure: characteristics of a novel plasmid type with low %G+C content. *Environ Microbiol* **11**: 937–949.
Hiasa, H., and Shea, M.E. (2000) DNA gyrase-mediated wrapping of the DNA strand is required for the replication fork arrest by the DNA gyrase–quinolone–DNA ternary complex. *J Biol Chem* **275:** 34780–34786.
Hirota, Y., Suzuki, H., Nishimura, Y., and Yasuda, S. (1977) On the process of cellular division in *Escherichia coli*: a mutant of *E. coli* lacking a murein-lipoprotein. *Proc Natl Acad Sci USA* **74:** 1417–1420.
Hoffman, L.R., D'Argenio, D.A., MacCoss, M.J., Zhang, Z., Jones, R.A., and Miller, S.I. (2005) Aminoglycoside antibiotics induce bacterial biofilm formation. *Nature* **436:** 1171–1175.
Hoffmeister, A., Ropolo, A., Vasseur, S., Mallo, G.V., Bodeker, H., Ritz-Laser, B., *et al.* (2002) The HMG-I/Y-related protein p8 binds to p300 and Pax2 *trans*-activation domain-interacting protein to regulate the *trans*-activation activity of the Pax2A and Pax2B transcription factors on the glucagon gene promoter. *J Biol Chem* **277:** 22314–22319.
Holden, M.T., McGowan, S.J., Bycroft, B.W., Stewart, G.S., Williams, P., and Salmond, G.P. (1998) Cryptic carbapenem antibiotic production genes are widespread in *Erwinia carotovora*: facile trans activation by the carR transcriptional regulator. *Microbiology* **144:** 1495–1508.
Horinouchi, S. (2007) Mining and polishing of the treasure trove in the bacterial genus *Streptomyces*. *Biosci Biotechnol Biochem* **71:** 283–299.
Ichimiya, T., Takeoka, K., Hiramatsu, K., Hirai, K., Yamasaki, T., and Nasu, M. (1996) The influence of azithromycin on the biofilm formation of *Pseudomonas aeruginosa in vitro*. *Chemotherapy* **42:** 186–191.
Kahan, J.S., Kahan, F.M., Goegelman, R., Currie, S.A., Jackson, M., Stapley, E.O., *et al.* (1972) Thienamycin, a new beta-lactam antibiotic. I. Discovery, taxonomy, isolation and physical properties. *J Antibiot (Tokyo)* **32:** 1–12.
Kato, J.Y., Funa, N., Watanabe, H., Ohnishi, Y., and Horinouchi, S. (2007) Biosynthesis of gamma-butyrolactone autoregulators that switch on secondary metabolism and morphological development in *Streptomyces*. *Proc Natl Acad Sci USA* **104:** 2378–2383.
Kaufmann, G.F., Sartorio, R., Lee, S.H., Rogers, C.J., Meijler, M.M., Moss, J.A., *et al.* (2005) Revisiting quorum sensing: discovery of additional chemical and biological functions for 3-oxo-*N*-acylhomoserine lactones. *Proc Natl Acad Sci USA* **102:** 309–314.
Kazimierczak, K.A., Scott, K.P., Kelly, D., and Aminov, R.I. (2009) Tetracycline resistome of the organic pig gut. *Appl Environ Microbiol* **75:** 1717–1722.
Keeney, D., Ruzin, A., and Bradford, P.A. (2007) RamA, a transcriptional regulator, and AcrAB, an RND-type efflux pump, are associated with decreased susceptibility to tigecycline in *Enterobacter cloacae*. *Microb Drug Resist* **13:** 1–6.
Keeney, D., Ruzin, A., McAleese, F., Murphy, E., and Brad-

ford, P.A. (2008) MarA-mediated overexpression of the AcrAB efflux pump results in decreased susceptibility to tigecycline in *Escherichia coli*. *J Antimicrob Chemother* **61:** 46–53.

Khan, N.H., Ishii, Y., Kimata-Kino, N., Esaki, H., Nishino, T., Nishimura, M., and Kogure, K. (2007) Isolation of *Pseudomonas aeruginosa* from open ocean and comparison with freshwater, clinical, and animal isolates. *Microb Ecol* **53:** 173–186.

Khokhlov, A.S., Tovarova, I.I., Borisova, L.N., Pliner, S.A., Shevchenko, L.N., Kornitskaia, E.I.A., *et al.* (1967) The A-factor, responsible for streptomycin biosynthesis by mutant strains of *Actinomyces streptomycini*. *Dokl Akad Nauk SSSR* **177:** 232–235.

Kim, S.C., and Carlson, K. (2006) Occurrence of ionophore antibiotics in water and sediments of a mixed-landscape watershed. *Water Res* **40:** 2549–2560.

Kim, S.R., Maenhaut-Michel, G., Yamada, M., Yamamoto, Y., Matsui, K., Sofuni, T., *et al.* (1997) Multiple pathways for SOS-induced mutagenesis in *Escherichia coli:* an overexpression of dinB/dinP results in strongly enhancing mutagenesis in the absence of any exogenous treatment to damage DNA. *Proc Natl Acad Sci USA* **94:** 13792–13797.

Knapp, C.W., Engemann, C.A., Hanson, M.L., Keen, P.L., Hall, K.J., and Graham, D.W. (2008) Indirect evidence of transposon-mediated selection of antibiotic resistance genes in aquatic systems at low-level oxytetracycline exposures. *Environ Sci Technol* **42:** 5348–5353.

Köhler, T., Dumas, J.L., and Van Delden, C. (2007) Ribosome protection prevents azithromycin-mediated quorum-sensing modulation and stationary-phase killing of *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **51:** 4243–4248.

Koike, S., Krapac, I.G., Oliver, H.D., Yannarell, A.C., Chee-Sanford, J.C., Aminov, R.I., and Mackie, R.I. (2007) Monitoring and source tracking of tetracycline resistance genes in lagoons and groundwater adjacent to swine production facilities over a 3-year period. *Appl Environ Microbiol* **73:** 4813–4823.

Krulwich, T.A., Jin, J., Guffanti, A.A., and Bechhofer, H. (2001) Functions of tetracycline efflux proteins that do not involve tetracycline. *J Mol Microbiol Biotechnol* **3:** 237–246.

Kuroda, H., Kuroda, M., Cui, L., and Hiramatsu, K. (2007) Subinhibitory concentrations of β-lactam induce haemolytic activity in *Staphylococcus aureus* through the SaeRS two-component system. *FEMS Microbiol Lett* **268:** 98–105.

Kurokawa, K., Itoh, T., Kuwahara, T., Oshima, K., Toh, H., Toyoda, A., *et al.* (2007) Comparative metagenomics revealed commonly enriched gene sets in human gut microbiomes. *DNA Res* **14:** 169–181.

Lavollay, M., Arthur, M., Fourgeaud, M., Dubost, L., Marie, A., Veziris, N., *et al.* (2008) The peptidoglycan of stationary-phase *Mycobacterium tuberculosis* predominantly contains cross-links generated by L,D-transpeptidation. *J Bacteriol* **190:** 4360–4366.

Lefort, A., Lafaurie, M., Massias, L., Petegnief, Y., Saleh-Mghir, A., Muller-Serieys, C., *et al.* (2003) Activity and diffusion of tigecycline (GAR-936) in experimental enterococcal endocarditis. *Antimicrob Agents Chemother* **47:** 216–222.

Leiva, M., Ruiz-Bravo, A., and Jimenez-Valera, M. (2008a) Effects of telithromycin in *in vitro* and *in vivo* models of lipopolysaccharide-induced airway inflammation. *Chest* **134:** 20–29.

Leiva, M., Ruiz-Bravo, A., Moreno, E., and Jiménez-Valera, M. (2008b) Telithromycin inhibits the production of proinflammatory mediators and the activation of NF-kappaB in *in vitro*-stimulated murine cells. *FEMS Immunol Med Microbiol* **53:** 343–350.

Lenski, R.E. (1997) The cost of antibiotic resistance – from the perspective of a bacterium. *Ciba Found Symp* **207:** 131–140.

Li, A., and Piel, J. (2002) A gene cluster from a marine *Streptomyces* encoding the biosynthesis of the aromatic spiroketal polyketide griseorhodin A. *Chem Biol* **9:** 1017–10126.

Lian, W., Jayapal, K.P., Charaniya, S., Mehra, S., Glod, F., Kyung, Y.S., *et al.* (2008) Genome-wide transcriptome analysis reveals that a pleiotropic antibiotic regulator, AfsS, modulates nutritional stress response in *Streptomyces Coelicolor* A3(2). *BMC Genomics* **29:** 56.

Liang, X., Yu C., Sun, J., Liu, H., Landwehr, C., Holmes, D., and Ji, Y. (2006) Inactivation of a two-component signal transduction system, SaeRS, eliminates adherence and attenuates virulence of *Staphylococcus aureus*. *Infect Immun* **74:** 4655–4665.

Lima-Bittencourt, C.I., Cursino, L., Gonçalves-Dornelas, H., Pontes, D.S., Nardi, R.M., Callisto, M., *et al.* (2007) Multiple antimicrobial resistance in *Enterobacteriaceae* isolates from pristine freshwater. *Genet Mol Res* **6:** 510–521.

Linares, J.F., Gustafsson, I., Baquero, F., and Martínez, J.L. (2006) Antibiotics as intermicrobial signaling agents instead of weapons. *Proc Natl Acad Sci USA* **103:** 19484–19489.

Liras, P., Gomez-Escribano, J.P., and Santamarta, I. (2008) Regulatory mechanisms controlling antibiotic production in *Streptomyces clavuligerus*. *J Ind Microbiol Biotechnol* **35:** 667–676.

Livermore, D.M. (1996) Are all beta-lactams created equal? *Scand J Infect Dis* **101:** 33–43.

Luo, N., Pereira, S., Sahin, O., Lin, J., Huang, S., Michel, L., and Zhang, Q. (2005) Enhanced *in vivo* fitness of fluoroquinolone-resistant *Campylobacter jejuni* in the absence of antibiotic selection pressure. *Proc Natl Acad Sci USA* **102:** 541–546.

McBride, M.J., and Baker, S.A. (1996) Development of techniques to genetically manipulate members of the genera *Cytophaga*, *Flavobacterium*, *Flexibacter*, and *Sporocytophaga*. *Appl Environ Microbiol* **62:** 3017–3022.

McGowan, S.J., Sebaihia, M., O'Leary, S., Hardie, K.R., Williams, P., Stewart, G.S.A.B., *et al.* (1997) Analysis of the carbapenem gene cluster of *Erwinia carotovora*: definition of the antibiotic biosynthetic genes and evidence for a novel β-lactam resistance mechanism. *Mol Microbiol* **26:** 545–556.

Macheboeuf, P., Contreras-Martel, C., Job, V., Dideberg, O., and Dessen, A. (2006) Penicillin binding proteins: key players in bacterial cell cycle and drug resistance processes. *FEMS Microbiol Rev* **30:** 673–691.

Maciá, M.D., Blanquer, D., Togores, B., Sauleda, J., Pérez, J.L., and Oliver, A. (2005) Hypermutation is a key factor

in development of multiple-antimicrobial resistance in *Pseudomonas aeruginosa* strains causing chronic lung infections. *Antimicrob Agents Chemother* **49:** 3382–3386.

Magnet, S., Bellais, S., Dubost, L., Fourgeaud, M., Mainardi, J.L., Petit-Frere, S., *et al.* (2007) Identification of the L,D-transpeptidases responsible for attachment of the Braun lipoprotein to *Escherichia coli* peptidoglycan. *J Bacteriol* **189:** 3927–3931.

Mainardi, J.L., Hugonnet, J.E., Rusconi, F., Fourgeaud, M., Dubost, L., Moumi, A.N., *et al.* (2007) Unexpected inhibition of peptidoglycan LD-transpeptidase from *Enterococcus faecium* by the β-lactam imipenem. *J Biol Chem* **282:** 30414–30422.

Maiques, E., Ubeda, C., Campoy, S., Salvador, N., Lasa, I., Novick, R.P., *et al.* (2006) β-Lactam antibiotics induce the SOS response and horizontal transfer of virulence factors in *Staphylococcus aureus*. *J Bacteriol* **188:** 2726–2729.

Martínez, J.L. (2008) Antibiotics and antibiotic resistance genes in natural environments. *Science* **321:** 365–367.

Martínez, J.L., Baquero, F., and Andersson, D.I. (2007) Predicting antibiotic resistance. *Nat Rev Microbiol* **5:** 958–965.

Martínez, J.L., Sánchez, M.B., Martínez-Solano, L., Hernandez, A., Garmendia, L., Fajardo, A., and Alvarez-Ortega, C. (2009) Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS Microbiol Rev* **33:** 430–449.

Mena, A., Smith, E.E., Burns, J.L., Speert, D.P., Moskowitz, S.M., Perez, J.L., and Oliver, A. (2008) Genetic adaptation of *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients is catalyzed by hypermutation. *J Bacteriol* **190:** 7910–7917.

Menendez, N., Nur-e-Alam, M., Brana, A.F., Rohr, J., Salas, J.A., and Mendez, C. (2004) Biosynthesis of the antitumor chromomycin A3 in *Streptomyces griseus*: analysis of the gene cluster and rational design of novel chromomycin analogs. *Chem Biol* **1:** 21–32.

Mercier, R.C., Kennedy, C., and Meadows, C. (2002) Antimicrobial activity of tigecycline (GAR-936) against *Enterococcus faecium* and *Staphylococcus aureus* used alone and in combination. *Pharmacotherapy* **22:** 1517–1523.

Meroueh, S.O., Minasov, G., Lee, W., Shoichet, B.K., and Mobashery, S. (2003) Structural aspects for evolution of beta-lactamases from penicillin-binding proteins. *J Am Chem Soc* **125:** 9612–9618.

Milatovic, D., Schmitz, F.J., Verhoef, J., and Fluit, A.C. (2003) Activities of the glycylcycline tigecycline (GAR-936) against 1924 recent European clinical bacterial isolates. *Antimicrob Agents Chemother* **47:** 400–404.

Miller, C., Thomsen, L.E., Gaggero, C., Mosseri, R., Ingmer, H., and Cohen, S.N. (2004) SOS response induction by beta-lactams and bacterial defense against antibiotic lethality. *Science* **305:** 1629–1631.

Moon, K., Shoemaker, N.B., Gardner, J.F., and Salyers, A.A. (2005) Regulation of excision genes of the *Bacteroides* conjugative transposon, CTnDOT. *J Bacteriol* **186:** 5732–5741.

Moonen, M.J.H., Fraaije, M.W., Rietjens, Y.M.C.M., Laane, C., and van Berkel, W.J.H. (2002) Flavoenzyme-catalyzed oxygenations and oxidations of phenolic compounds. *Adv Synth Catal* **344:** 1–13.

Moore, I.F., Hughes, D.W., and Wright, G.D. (2005) Tigecycline is modified by the flavin-dependent monooxygenase TetX. *Biochemistry* **44:** 11829–111835.

Morimura, T., Hashiba, M., Kameda, H., Takami, M., Takahama, H., Ohshige, M., and Sugawara, F. (2008) Identification of macrolide antibiotic-binding Human_p8 protein. *J Antibiot (Tokyo)* **61:** 291–296.

Nannini, E.C., Pai, S.R., Singh, K.V., and Murray, B.E. (2003) Activity of tigecycline (GAR-936), a novel glycylcycline, against enterococci in the mouse peritonitis model. *Antimicrob Agents Chemother* **47:** 529–532.

Nicolau DP. (2008) Carbapenems: a potent class of antibiotics. *Expert Opin Pharmacother* **9:** 23–37.

Nilsson, A.I., Zorzet, A., Kanth, A., Dahlström, S., Berg, O.G., and Andersson, D.I. (2006) Reducing the fitness cost of antibiotic resistance by amplification of initiator tRNA genes. *Proc Natl Acad Sci USA* **103:** 6976–6981.

Nishida, H., Ohnishi, Y., Beppu, T., and Horinouchi, S. (2007) Evolution of gamma-butyrolactone synthases and receptors in *Streptomyces*. *Environ Microbiol* **9:** 1986–1994.

Nord, C.E., Sillerström, E., and Wahlund, E. (2006) Effect of tigecycline on normal oropharyngeal and intestinal microflora. *Antimicrob Agents Chemother* **50:** 3375–3380.

Novakova, R., Homerova, D., Feckova, L., and Kormanec, J. (2005) Characterization of a regulatory gene essential for the production of the angucycline-like polyketide antibiotic auricin in *Streptomyces aureofaciens* CCM 3239. *Microbiology* **151:** 2693–2706.

Núñez, L., Méndez, C., Braña, A., Blanco, G., and Salas, J. (2003) The biosynthetic gene cluster for the β-lactam carbapenem thienamycin in *Streptomyces cattleya*. *Chem Biology* **10:** 301–311.

Parker, W.L., Rathnum, M.L., Wells, J.S., Jr, Trejo, W.H., Principe, P.A., and Sykes, R.B. (1982) SQ 27,860, a simple carbapenem produced by species of *Serratia* and *Erwinia*. *J Antibiot (Tokyo)* **35:** 653–660.

Pei, R., Kim, S.C., Carlson, K.H., and Pruden, A. (2006) Effect of river landscape on the sediment concentrations of antibiotics and corresponding antibiotic resistance genes (ARG). *Water Res* **40:** 2427–2435.

Peleg, A.Y., Adams, J., and Paterson, D.L. (2007) Tigecycline efflux as a mechanism for nonsusceptibility in *Acinetobacter baumannii*. *Antimicrob Agents Chemother* **51:** 2065–2069.

Petersen, P.J., Jacobus, N.V., Weiss, W.J., Sum, P.E., and Testa, R.T. (1999) *In vitro* and *in vivo* antibacterial activities of a novel glycylcycline, the 9-t-butylglycylamido derivative of minocycline (GAR-936). *Antimicrob Agents Chemother* **43:** 738–744.

Petersen, P.J., Bradford, P.A., Weiss, W.J., Murphy, T.M., Sum, P.E., and Projan, S.J. (2002) *In vitro* and *in vivo* activities of tigecycline (GAR-936), daptomycin, and comparative antimicrobial agents against glycopeptide-intermediate *Staphylococcus aureus* and other resistant gram-positive pathogens. *Antimicrob Agents Chemother* **46:** 2595–2601.

Piddock, L.J. (2006) Multidrug-resistance efflux pumps – not just for resistance. *Nat Rev Microbiol* **4:** 629–636.

Poirel, L., Rodriguez-Martinez, J.M., Mammeri, H., Liard, A., and Nordmann, P. (2005) Origin of plasmid-mediated quinolone resistance determinant QnrA. *Antimicrob Agents Chemother* **49:** 3523–3525.

Prado, L., Fernandez, E., Weissbach, U., Blanco, G., Quiros, L.M., Brana, A.F., *et al.* (1999) Oxidative cleavage of premithramycin B is one of the last steps in the biosynthesis of the antitumor drug mithramycin. *Chem Biol* **6:** 19–30.

Ramadhan, A.A., and Hegedus, E. (2005) Survivability of vancomycin resistant enterococci and fitness cost of vancomycin resistance acquisition. *J Clin Pathol* **58:** 744–746.

Rigali, S., Titgemeyer, F., Barends, S., Mulder, S., Thomae, A.W., Hopwood, D.A., and van Wezel, G.P. (2008) Feast or famine: the global regulator DasR links nutrient stress to antibiotic production by *Streptomyces*. *EMBO Rep* **9:** 670–675.

Roberts, M.C. (2005) Update on acquired tetracycline resistance genes. *FEMS Microbiol Lett* **245:** 195–203.

Robillard, N.J., Tally, F.P., and Malamy, M.H. (1985) Tn*4400*, a compound transposon isolated from *Bacteroides fragilis*, functions in *Escherichia coli*. *J Bacteriol* **164:** 1248–1255.

Rodríguez, M., Núñez, L.E., Braña, A.F., Méndez, C., Salas, J.A., and Blanco, G. (2008) Identification of transcriptional activators for thienamycin and cephamycin C biosynthetic genes within the thienamycin gene cluster from *Streptomyces cattleya*. *Mol Microbiol* **69:** 633–645.

Rogasch, K., Rühmling, V., Pané-Farré, J., Höper, D., Weinberg, C., Fuchs, S., *et al.* (2006) Influence of the two-component system SaeRS on global gene expression in two different *Staphylococcus aureus* strains. *J Bacteriol* **188:** 7742–7758.

Ruzin, A., Visalli, M.A., Keeney, D., and Bradford, P.A. (2005) Influence of transcriptional activator RamA on expression of multidrug efflux pump AcrAB and tigecycline susceptibility in *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* **49:** 1017–1022.

Sánchez, M.B., Hernández, A., Rodríguez-Martínez, J.M., Martínez-Martínez, L., and Martínez, J.L. (2008) Predictive analysis of transmissible quinolone resistance indicates *Stenotrophomonas maltophilia* as a potential source of a novel family of Qnr determinants. *BMC Microbiol* **8:** 148.

Shiner, E.K., Rumbaugh, K.P., and Williams, S.C. (2005) Inter-kingdom signaling: deciphering the language of acyl homoserine lactones. *FEMS Microbiol Rev* **29:** 935–947.

Shoemaker, N.B., Guthrie, E.P., Salyers, A.A., and Gardner, J.F. (1985) Evidence that the clindamycin-erythromycin resistance gene of *Bacteroides* plasmids pBF4 is on a transposable element. *J Bacteriol* **162:** 626–632.

Showsh, S.A., and Andrews, R.E., Jr (1992) Tetracycline enhances Tn*916*-mediated conjugal transfer. *Plasmid* **28:** 213–224.

Skindersoe, M.E., Alhede, M., Phipps, R., Yang, L., Jensen, P.O., Rasmussen, T.B., *et al.* (2008) Effects of antibiotics on quorum sensing in *Pseudomonas aeruginosa*. *Antimicrob Agents Chemother* **52:** 3648–3663.

Slater, H., Crow, M., Everson, L., and Salmond, G.P. (2003) Phosphate availability regulates biosynthesis of two antibiotics, prodigiosin and carbapenem. *Serratia* via both quorum-sensing-dependent and -independent pathways. *Mol Microbiol* **47:** 303–320.

Smith, E.E., Buckley, D.G., Wu, Z., Saenphimmachak, C., Hoffman, L.R., D'Argenio, D.A., *et al.* (2006) Genetic adaptation by *Pseudomonas aeruginosa* to the airways of cystic fibrosis patients. *Proc Natl Acad Sci USA* **103:** 8487–8492.

Song, J.S., Jeon, J.H., Lee, J.H., Jeong, S.H., Jeong, B.C., Kim, S.J., *et al.* (2005) Molecular characterization of TEM-type beta-lactamases identified in cold-seep sediments of Edison Seamount (south of Lihir Island, Papua New Guinea). *J Microbiol* **43:** 172–178.

Song, B., Wang, G.R., Shoemaker, N.B., and Salyers, A.A. (2009) An unexpected effect of tetracycline concentration: growth phase-associated excision of the *Bacteroides* mobilizable transposon NBU1. *J Bacteriol* **191:** 1078–1082.

Speer, B.S., Bedzyk, L., and Salyers, A.A. (1991) Evidence that a novel tetracycline resistance gene found on two *Bacteroides* transposons encodes an NADP-requiring oxidoreductase. *J Bacteriol* **173:** 176–183.

Stevens, A.M., Shoemaker, N.B., Li, L.Y., and Salyers, A.A. (1993) Tetracycline regulation of genes on *Bacteroides* conjugative transposons. *J Bacteriol* **175:** 6134–6141.

Strachan, D.P. (1989) Hay fever, hygiene, and household size. *BMJ* **299:** 1259–1260.

Takano, E. (2006) γ-Butyrolactones: *Streptomyces* signalling molecules regulating antibiotic production and differentiation. *Curr Opin Microbiol* **9:** 287–294.

Tamaoki, J., Kadota, J., and Takizawa, H. (2004) Clinical implications of the immunomodulatory effects of macrolides. *Am J Med* **117** (Suppl. 9A)**:** 5S–11S.

Tateda, K., Standiford, T.J., Pechere, J.C., and Yamaguchi, K. (2004) Regulatory effects of macrolides on bacterial virulence: potential role as quorum-sensing inhibitors. *Curr Pharm Des* **10:** 3055–3065.

Thomson, N.R., Crow, M.A., McGowan, S.J., Cox, A., and Salmond, G.P. (2000) Biosynthesis of carbapenem antibiotic and prodigiosin pigment in *Serratia* is under quorum sensing control. *Mol Microbiol* **36:** 539–556.

Torres, O.R., Korman, R.Z., Zahler, S.A., and Dunny, G.M. (1991) The conjugative transposon Tn*925*: enhancement of conjugal transfer by tetracycline in *Enterococcus faecalis* and mobilization of chromosomal genes in *Bacillus subtilis* and *E. faecalis*. *Mol Gen Genet* **225:** 395–400.

Tran, J.H., and Jacoby, G.A. (2002) Mechanism of plasmid-mediated quinolone resistance. *Proc Natl Acad Sci USA* **99:** 5638–5642.

Tran, J.H., Jacoby, G.A., and Hooper, D.C. (2005) Interaction of the plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. *Antimicrob Agents Chemother* **49:** 118–125.

Tsui, W.H., Yim, G., Wang, H.H., McClure, J.E., Surette, M.G., and Davies, J. (2004) Dual effects of MLS antibiotics: transcriptional modulation and interactions on the ribosome. *Chem Biol* **11:** 1307–1316.

Ubeda, C., Maiques, E., Knecht, E., Lasa, I., Novick, R.P., and Penadés, J.R. (2005) Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. *Mol Microbiol* **56:** 836–844.

Valentine, P.J., Shoemaker, N.B., and Salyers, A.A. (1988) Mobilization of *Bacteroides* plasmids by *Bacteroides* conjugal elements. *J Bacteriol* **170:** 1319–1324.

Visalli, M.A., Murphy, E., Projan, S.J., and Bradford, P.A. (2003) AcrAB multidrug efflux pump is associated with reduced levels of susceptibility to tigecycline (GAR-936) in *Proteus mirabilis*. *Antimicrob Agents Chemother* **47:** 665–669.

Wallace, R.J., Jr, Brown-Elliott, B.A., Crist, C.J., Mann, L., and Wilson, R.W. (2002) Comparison of the *in vitro* activity of the glycylcycline tigecycline (formerly GAR-936) with those of tetracycline, minocycline, and doxycycline against isolates of nontuberculous mycobacteria. *Antimicrob Agents Chemother* **46:** 3164–3167.

Wang, Y.J., and Leadbetter, J.R. (2005) Rapid acyl-homoserine lactone quorum signal biodegradation in diverse soils. *Appl Environ Microbiol* **71:** 1291–1299.

Weber, T., Welzel, K., Pelzer, S., Vente, A., and Wohlleben, W. (2003) Exploiting the genetic potential of polyketide producing streptomycetes. *J Biotechnol* **106:** 221–232.

Webster, G., and Del Rosso, J.Q. (2007) Anti-inflammatory activity of tetracyclines. *Dermatol Clin* **25:** 133–135.

Wei, Y., and Bachhofer, D.H. (2002) Tetracycline induces stabilization of mRNA in *Bacillus subtilis*. *J Bacteriol* **184:** 889–894.

Weinberg, J.M. (2005) The anti-inflammatory effects of tetracyclines. *Cutis* **75:** 6–11.

Whittle, G., Hund, B.D., Shoemaker, N.B., and Salyers, A.A. (2001) Characterization of the 13-kilobase ermF region of the *Bacteroides* conjugative transposon CTnDOT. *Appl Environ Microbiol* **67:** 3488–3495.

Whittle, G., Shoemaker, N.B., and Salyers, A.A. (2002) Identification of two genes involved in the modulation of conjugal transfer of the *Bacteroides* conjugative transposon CTnDOT. *J Bacteriol* **184:** 3839–3847.

Williamson, J.M., Inamine, E., Wilson, K.E., Douglas, A.W., Liesch, J.M., and Albers-Schonberg, G. (1985) Biosynthesis of the beta-lactam antibiotic, thienamycin, by *Streptomyces cattleya*. *J Biol Chem* **260:** 4637–4647.

Wright, G.D. (2007) The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat Rev Microbiol* **5:** 175–186.

Yang, S., and Carlson, K. (2003) Evolution of antibiotic occurrence in a river through pristine, urban and agricultural landscapes. *Water Res* **37:** 4645–4656.

Yang, W., Moore, I.F., Koteva, K.P., Bareich, D.C., Hughes, D.W., and Wright, G.D. (2004) TetX is a flavin-dependent monooxygenase conferring resistance to tetracycline antibiotics. *J Biol Chem* **279:** 52346–52352.

Yim, G., Wang, H.H., and Davies, J. (2006) The truth about antibiotics. *Int J Med Microbiol* **296:** 163–170.

Yuhas, Y., Berent, E., Cohen, R., and Ashkenazi, S. (2008) Role of NF-{kappa}B Activation and PPAR{gamma} inhibition in the effect of rifampin on inducible nitric oxide synthase transcription in human lung epithelial cells. *Antimicrob Agents Chemother* **53:** 1539–1545.