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REVIEW ARTICLE

Emergence and spread of antibiotic resistance: setting a parameter space

JOSÉ LUIS MARTÍNEZ[1] & FERNANDO BAQUERO[2]

[1]*Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología, CSIC, Darwin 3, Cantoblanco, 28049, Madrid, Spain, and* [2]*Servicio de Microbiología, Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS) and CIBER Epidemiología y Salud Pública (CIBERESP), Madrid, Spain*

Abstract

The emergence and spread of antibiotic resistance among human pathogens is a relevant problem for human health and one of the few evolution processes amenable to experimental studies. In the present review, we discuss some basic aspects of antibiotic resistance, including mechanisms of resistance, origin of resistance genes, and bottlenecks that modulate the acquisition and spread of antibiotic resistance among human pathogens. In addition, we analyse several parameters that modulate the evolution landscape of antibiotic resistance. Learning why some resistance mechanisms emerge but do not evolve after a first burst, whereas others can spread over the entire world very rapidly, mimicking a chain reaction, is important for predicting the evolution, and relevance for human health, of a given mechanism of resistance. Because of this, we propose that the emergence and spread of antibiotic resistance can only be understood in a multi-parameter space. Measuring the effect on antibiotic resistance of parameters such as contact rates, transfer rates, integration rates, replication rates, diversification rates, and selection rates, for different genes and organisms, growing under different conditions in distinct ecosystems, will allow for a better prediction of antibiotic resistance and possibilities of focused interventions.

Key words: *Antibiotic resistance, evolution, horizontal gene transfer, resistome*

Introduction

Antibiotics are likely the most successful drug category in use for improvements of human health. Their introduction for treatment of human infections allowed for an impressive increase in life expectancies to the point that, in 1967, William Stewart, the Surgeon-General of the United States of America, stated: 'The time has come to close the book on infectious diseases' (1). Unfortunately that was not true. Infections remain among the major causes of human mortality and morbidity. One of the reasons for this situation is the fast adaptation of the organisms to antibiotics; indeed, resistance arose quite early after antibiotics were introduced for therapy (2). Besides treating infections in the community, the use of antibiotics allowed the widespread implementation of hospital therapeutic practices such as immunosuppression associated with transplantation or with anticancer therapy, extensive surgery, and even catheterization. These patients have an extremely high risk of acquiring an infection, which means that these techniques can be safely used only when accurate methods for preventing or treating infections are available.

Acquisition of resistance by human pathogens may thus compromise not just the treatment of infectious diseases but also the implementation of several clinical practices that are now taken for granted provided there are good anti-infectious agents (3,4). One relevant question then concerns the origin of resistance and the mechanisms for its dissemination. Resistance to antibiotics can be developed either by mutations (5) or by the acquisition, through

Correspondence: José Luis Martínez, Departamento de Biotecnología Microbiana, Centro Nacional de Biotecnología, CSIC, Darwin 3, Cantoblanco, 28049, Madrid, Spain. E-mail: jlmtnez@cnb.csic.es

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horizontal gene transfer (HGT), of resistance genes (6–8). Since bacterial pathogens are by definition susceptible to commercial antibiotics before they are used for therapy (compounds for which pathogens are resistant should not be considered as antibiotics irrespective of whether or not they kill non-pathogens), resistance genes should come from somewhere else. The origin of antibiotic resistance genes may be on commensal (non-pathogenic) (9) or on environmental microbiota (10). This means that if we want to understand in full the cycle of acquisition and spread of antibiotic resistance among bacterial human pathogens, these ecosystems should be taken into consideration (11–14). In the present article, we will review current information on the different mechanisms of antibiotic resistance, on the origin of resistance genes, and on the bottlenecks that may modulate acquisition and spread of antibiotic resistance. This analysis provides an image of the complexity of the evolutionary field in which antibiotic resistance is spreading, and suggests a number of data (rates) that will be required to construct a parameter space for a proper understanding and eventually prediction of antibiotic resistance.

Mechanisms of antibiotic resistance

Bacterial growth inhibition by an antibiotic is achieved when the antimicrobial efficiently interacts with its target. For this interaction to occur, there are just two relevant elements: the antibiotic must recognize the target, and the concentration of the antibiotic at the target must be enough for achieving an efficient inhibition of its activity. All mechanisms of resistance are then based on either modifying the target or reducing the concentration of free antibiotic that can access the target. To interact with their target, antibiotics are required to traverse different bacterial envelopes and occasionally be activated by a cellular enzyme as it happens for isoniazid (15). Mutations in genes coding for transporters, targets, or proteins activating the pre-antibiotic can confer resistance (13). These mechanisms do not affect the active antibiotic itself and can then be considered as 'passive mechanisms of resistance'. Usually, transfer of mutated genes by HGT does not confer resistance [an exception being mutations of topoisomerases in *Streptococcus pneumoniae* (16,17)], which means that the major element driving the spread of mutation-acquired antibiotic resistance is clonal expansion.

In addition to these mechanisms, resistance can be achieved by reducing the amount of active antibiotic, either because of its modification by antibiotic-inactivating enzymes or by its efflux through multidrug efflux pumps. These elements can be considered as 'active mechanisms of resistance', and their introduction in another host can confer resistance; this means that this type of resistance can spread either by clonal expansion or by HGT (18). Elements that modify the target or protect it from the action of antibiotics, as the Qnr quinolone resistance determinants (19), can also be considered as 'active mechanisms of resistance' that can also be spread through HGT.

In addition to these classical antibiotic resistance determinants, recent work has shown that several elements, involved in basic processes of bacterial metabolism, may modulate susceptibility to antimicrobials (20–27).

Origin of resistance genes

Comparisons of plasmids from pre- and post-antibiotic eras have shown that bacterial populations have acquired resistance genes not present before in human pathogens (28). One hypothesis for explaining the origin of such genes was raised some decades ago by Julian Davies. Most antimicrobials currently in use are produced by environmental micro-organisms or are derivatives of these natural antibiotics (29). These producers must have systems to avoid the activity of the antibiotics they produce, and these elements can be resistance genes upon their transfer to human pathogens. Inspection of the genomes of producers has shown that they contain genes belonging to the same families as resistance genes currently present in populations of human bacterial pathogens (30). However, to date none of these genes present in producers has been found in human pathogens. Notably, in the only two cases in which the origin of resistance has been accurately tracked, namely *qnrA* from *Shewanella algae* (31) and $bla_{\text{CTX-M}}$ from *Kluyvera* (32,33), the original organisms, where these genes evolved, were not antibiotic producers.

Different genomic and functional metagenomic works have shown that indeed genes which can confer resistance upon expression in an heterologous host are found in any studied micro-organism and ecosystem (9,30,34). One question that remains is the original function of resistance genes (11,13). Whereas their role as detoxification elements in the producers is quite clear, their function in non-producers is less well understood. One can argue that these elements have been selected to avoid the activity of the antibiotics produced by surrounding organisms. However, most *Enterobacteriaceae* harbour chromosomally encoded AmpC beta-lactamases (35), but the presence of beta-lactam producers in the gut has never been described. Following a similar reasoning, it is difficult to support the idea that several

multi-drug efflux pumps that extrude quinolones or the quinolone resistance proteins Qnrs (36,37) were born to avoid the activity of quinolones—synthetic compounds that were not present in nature until the 1960s.

Even in the case of antibiotic producers the functional role of these determinants is detoxification of antibiotics they produce rather than resistance to antibiotics produced by other micro-organisms. One article indicates, however, that co-evolution of production and counter-resistance may occur in soil (38), but even taking this issue into consideration several genes, which can confer resistance upon their transfer to a new host, have different functional roles in the original host organism. One example is the chromosomal aminoglycoside-inactivating enzyme of *Providencia stuartii* (39,40). This enzyme is involved in the O-acetylation of *P. stuartii* peptidoglycan. However, its substrate is very similar to gentamycin, which means it can inactivate this antibiotic and hence confer resistance. The same argument applies for multi-drug resistance (MDR) efflux pumps; they are encoded in all chromosomes of all organisms, and they can extrude a large variety of compounds, including antibiotics. For instance, AcrAB, which is the most important MDR efflux pump in *Enterobacteriaceae*, can extrude bile salts (41), and its activity is required to colonize the gut (42,43). The homologues of this efflux pump are also involved in the bacterial wilt virulence of *Ralstonia solanacearum* (44). Besides contributing to bacterial–host interactions, MDR efflux pumps may be involved in intercellular signalling. This is the case of *Pseudomonas aeruginosa*, whose efflux pumps can contribute, in addition to antibiotic resistance, to extrusion of quorum-sensing signals (45–48).

It has been suggested that genes not involved in resistance in their original hosts should be considered as pre-resistance genes, and they require further evolution to confer a full resistance phenotype. This is not always true. Introduction of different alleles of the QnrA-encoding gene present in the chromosome of *S. algae* confers a similar level of resistance as the plasmid-encoded QnrA [(49), J.L.M. unpublished paper]. Furthermore, some genes that do not confer resistance in their original host, because their level of expression is very low, can confer antibiotic resistance (without any mutation) when present in high copy-number plasmids (50,51) or when their expression is triggered by a strong promoter (52). Altogether, this indicates that a gene that does not evolve in its original host to confer resistance can be a relevant resistance gene once it is transferred to a human pathogen (13). This mechanism of evolution in which the function of an element changes, without any genetic change, but just because of a change of environment has been named exaptation (53) and is fundamental to understand the first step in the acquisition of resistance by human pathogens. The reasons why an enzyme or an efflux pump can confer resistance to antibiotics rely on their substrates' specificity. In principle, any enzyme (or efflux pump) with a substrate similar to an antibiotic used for therapy might confer resistance, no matter its function in the original host. This situation expands the range of micro-organisms that can eventually be a source of antibiotic resistance to nearly all microbiota. However, the number of resistance elements currently present in human pathogens is low in comparison, which means that there are bottlenecks in the acquisition and spread of resistance genes among pathogenic micro-organisms (54).

Bottlenecks in the acquisition and spread of antibiotic resistance

The use and misuse of antibiotics have caused the enrichment of a small subset of antibiotic resistance genes among all that can be found in natural microbiota, not only in clinical settings but in environmental ecosystems as well (54–56). The first bottleneck to explain such an enrichment is the ecological connectivity; for example, it is very unlikely that a resistance gene present in a bacterium that only grows at the deep earth sub-surface will be transferred to a human pathogen. The same situation applies for gene exchange communities; belonging to these communities will increase the probabilities of transfer of a resistance element among the members of this microbiota (57). The second bottleneck in the acquisition of a resistance gene is the presence in the community of a previously acquired resistance gene, which can be denoted the founder effect. If a resistance gene is already spread in the community, the presence of the antibiotic for which such a gene confers resistance will not inhibit growth of bacterial carriers. Thus, in the absence of selective pressure, novel resistance genes against the same antibiotic will not be acquired. A third bottleneck, in this case for the spread of a given resistance gene, consists of fitness costs (58). The acquisition of resistance can confer a fitness cost that sometimes is specific for the involved gene/mutation (46,49,59). Only those resistance elements presenting affordable fitness costs, or for which compensatory mutations are easily achieved (60), will be maintained in the populations (61). Maintenance, and consequently spread of resistance, can be enhanced if the resistance gene is associated with other determinants that can be co-selected, such as other resistance genes, virulence determinants, or elements conferring an ecological advantage.

We can then see that there have been two ages in the evolution of resistance (62). Before the use of antibiotics by humans, several elements, which eventually can confer resistance, have evolved for several millions of years in the genomes of the microorganisms. The original function of these genes is not necessarily resistance. After the onset of use of antibiotics, some genes have been transferred into new hosts (human pathogens) in which their unique role is conferring resistance. In addition, the strong antibiotic selection exerted on these acquired resistance genes is likely to drive their further diversification. A good illustration of this process is the evolution of the family of TEM beta-lactamases after the introduction of beta-lactamase inhibitors and novel cephalosporins (63–66).

The complex effects of the anthropogenic use of antibiotics on bacterial biology

The use and misuse of antibiotics for human and animal therapy and as growth promoters in farms have produced an increased selection of antibiotic-resistant pathogens. Most studies on this topic have dealt with human/animal pathogens. However, antibiotics and bacterial pathogens containing resistance genes are constantly released into natural ecosystems, and the effects they may have in such environments are not fully understood.

It was early stated that antibiotics have an inhibitory role in natural ecosystems (67). In this context, resistance genes would have emerged to counteract such effects. However, recent work suggests that, at the low concentrations present in natural ecosystems, antibiotics may have other natural functions, not necessarily linked with killing competitors, but instead being involved in cell-to-cell signalling networks (68–73). Conversely, different studies have shown that resistance genes, acquired by human pathogens, may have a primary different function in their original hosts (11,74). The anthropogenic production of antibiotics, and their release at high concentrations in the microbiosphere, results in a change of their functional role, from the natural signalling function towards growth inhibition. That situation produces a disturbance of the microbial environmental networks, antibiotic resistance tending to preserve their integrity. Such adaptation is linked to the emergence and dissemination of antibiotic resistance genes and of all genetic and cellular vehicles in which these genes are located (55,56). Selection mediated by the phenotype produced by a particular gene-resistant variant in a bacterial clone means the selection of all genes of the genome of such clones, but also selection of the mobile genetic elements and platforms contained in these clones and all the genes they contain. Selection of particular mobile elements implies an increased possibility of spread of these elements to other clones (and species) which, in turn, are selected with all their genes and mobile elements. Note that selection of a resistant clone and their complex of mating associates forming genetic exchange communities (57,75) might result in changes in the microbiome structure. In fact the initial selection of antibiotic resistance might behave as starter (ignition point) of a 'chain reaction', particularly in antibiotic-polluted environments. The trajectory of such a reaction is difficult to predict, as each selective step is combined with changing micro-ecological landscapes, locally shaping the population biology of antibiotic resistance, and thereby generating different evolutionary units, from genes to integrons, transposons, plasmids, clones, species, to bacterial communities and microbiotic ensembles (76). In addition to modifying the frequency and the interactive field of each of these units, antibiotics might increase the number and evolvability of clinically relevant antibiotic resistance genes, but probably also of many other genes with different primary functions but with a resistance phenotype present in the environmental resistome. In this regard, it is important to mention the multilevel selection pressure of antibiotics (76). They influence the abundance, modularity, and spread of integrons, transposons, and plasmids, mostly acting on structures present before the antibiotic era, and enrich particular bacterial lineages and clones and contribute to local clonalization processes. Finally, antibiotics amplify particular genetic exchange communities sharing antibiotic resistance genes and platforms within microbiomes. In summary, exposure to antibiotics has a multi-hierarchical influence on the bacterial world. The challenge is how to measure the effects of antibiotic exposure to predict and hopefully to prevent deep and potentially harmful effects on the ecology of public health. For that purpose we need to establish a space of composite parameters.

A space of composite parameters determining the emergence and spread of antibiotic resistance

Antibiotic resistance evolves and disseminates in a complex parameter space. This space is determined by a limited set of axes determining all possible combinations of values for all different parameters (77). Different regions of the parameter space produce different types of local behaviour, expressed as families of probability distributions. The definition and quantification of parameters involved in antibiotic

resistance remain some of the most urgent challenges of current research in public health. An important problem is the possible multiplicity of parametric spaces depending on the different evolutionary units at different hierarchical levels, as genes present in plasmids, and plasmids in bacterial clones (18).

Considering this complexity, and as a first approach, we can qualitatively distinguish the following composite parameters: 1) *contact rates*; this set of parameters refers to the probability that two particular evolutionary units could be *in close contact* during a sufficient period of time, enabling potential interactions; 2) *transfer rates*; this set of parameters refers to the probability that one evolutionary unit *moves into* another unit of the same or different hierarchical level; 3) *integration rates*; this set of parameters refers to the probability that one transferred unit could be *stably maintained in coexistence* with another unit or assembled with other units; 4) *replication rates*; this set of parameters refers to the probability that a particular unit *will increase in copy number* at a certain speed and reaching certain final densities; 5) *diversification rates*; this set of parameters refers to the probability that a particular unit produces *genetic variant units* at certain rates, and variants of these variants; and 6) *selection rates*; this final set of parameters refers to the probability that a particular unit might be *replicating differentially than other units* of the same hierarchical level as the result of the carriage of genes providing higher fitness. Note that *active selection* of a higher-unit level might result in *passive selection* of lower units integrated in the former one.

The parametric space resulting from the above set of six rates measuring interactions is certainly modified (even determined) by another group of parameters, the *ecological parameters*. These are environmental parameters whose changes might influence the above-mentioned rates. Among these parameters we can mention: density of colonized and colonizable hosts; population sizes of bacteria per host during colonization and infection; susceptibility to colonization of hosts, including age, nutrition, illness-facilitated colonization; frequency of between-hosts interactions (such as animal–human interaction); host natural and acquired immune response to colonizing organisms; ecological parameters of colonizable areas, including interaction with local microbiota and frequency and type of antibiotic-resistant commensals; migration and dispersal of colonized hosts; antibiotic exposure; overall density of antibiotic use, type of antibiotics and mode of action, dosage and duration of therapy, adherence to therapy, selective concentrations, antibiotic combinations; mode of transmission of resistant organisms; transmission rates between hosts (antibiotic treated and not-treated, infected, and not-infected); time of contact between hosts; exposure to biocides; hygiene, infection control, sanitation; food, drinking-water and water body contamination, and host exposure; and environmental contamination by resistant organisms in soil, including sewage and water bodies.

A closer view of factors determining the parameters influencing emergence and spread of antibiotic resistance

In this section we will enumerate a number of factors (sub-parameters), which should serve to provide a complex quantitative value of the main parameters defined in the previous section. The first main parameter is *contact rate*, which obviously depends on the population size of the elements entering in interaction. Therefore, the contact rates parameter should be proportional (for a given environment) to the absolute number of bacterial cells of a given bacterial clone (donors or recipients of antibiotic resistance), the number of bacterial cells of its clonal complex (kin-related groups of clones), the number of bacterial cells of the corresponding phylogroup, species, family, or the number of bacterial cells, which are part of the same genetic exchange community. Beyond bacteria–bacteria interactions, the exploration of contact rates should include the frequency of interactions between bacterial cells (donors or recipients of antibiotic resistance) and colonizable areas of human or animal individuals, bacterial cells and contact areas of individuals (such as hands; contact rates is a critical parameter in hospital infection; (78);). Contact rates should also be explored in bacterial cells and colonizable environment (water, soil, food). In summary, the local absolute amount of cells (resistant and potential recipients of resistance) is a critical parameter. Most importantly, the net result of an epidemic involving an antibiotic-resistant high-risk clone is the overall increase of the interactions (contacts) that the resulting abundance of the cells of this clone is triggering. Of course factors such as sanitation, hygienic containment, density of colonizable areas, and even climate will influence the cell density and hence contact rates. Indeed, these factors are probably critical in the emergence and spread of antibiotic resistance in crowded farms, and/or underdeveloped warm countries with poor hygiene standards. Contact rates between animal, human, and environmental bacteria in sewage and polluted soil create efficient 'biological interactive reactors' for the building up and evolution of antibiotic resistance (79).

The second main parameter is *transfer rates*. Interaction between cells (contact rates, previous paragraph) is a necessary condition, but not a

sufficient one for acquisition of antibiotic resistance traits. The interaction only influences the spread (and possibly evolution) of antibiotic resistance if genetic transfer occurs. The overall transferability of the resistant trait will be influenced by the rate of transfer of: 1) plasmids, integrative-conjugative elements, or chromosomal fragments (including islands) into recipient bacterial cells; 2) transfer of plasmid resistance genes into another plasmid, or into the chromosome; 3) chromosomal genes into plasmids; 4) transposons into plasmids or the chromosome. These transfer rates can vary depending on whether transfer occurs between cells of the same clone, clonal complex, phylogroup, species, members of genetic exchange communities, or higher taxa. Of course transfer rates might be correlated with other factors, such as growth rate, and co-operative (pheromones, co-aggregation, synergistic co-localization) or antagonistic activities (bacteriocins, microcins, bacteriophages). Different plasmid types are able to be transferred with different efficiency, some of them with a broad-host range, and a number of plasmids are able to mobilize non-conjugative plasmids. Because of the density- and frequency-dependent nature of HGT, transfer rates depend on these factors. At a higher level in the hierarchy, we should consider transfer rates of bacterial clones into secondary hosts (such as in hospital infection, or humans to animals or vice versa), or transfer of bacterial microbiota into secondary hosts (such as from mother to child). Transfer rates to secondary hosts might differ according to the type of host. For antibiotic resistance, mainly whether the secondary host is or is not under antibiotic therapy, with one or more drugs (80,81), as well as dosages and intervals between doses (82). Eventually, there are differences among clones of the same species in their transfer ability.

The third main parameter deals with *integration rates*. Contact between donors and recipient elements and transfer of resistant determinants (genes and their vehicles) do not assure a long-term association of resistance in particular hosts. Note that the term 'integration' is understood here not as an outcome of recombination processes in molecular genetics, but rather as in software-engineering environments; that is, not as a property of a single element, but of its relationships with other elements of the recipient platforms: the degree of integration refers to the extent to which tools agree (83). In this sense, the concept of integration is close to that of maintenance, and excision or loss is negative integration. Generally, phylogenetic closeness tends to increase HGT and stable integration, but also ecologically compatible (integrated) communities of phylogenetically distant organisms can be linked by HGT (84). In that sense, 'integration rates' measure rates of maintenance of plasmids or integrative-conjugative elements into bacterial cells, with or without CRISPR immunity (81). Similarly, we can consider integration rates of chromosomal fragments into genomes of other cells (recombination), plasmid genes into another plasmid, plasmid coexistence with other plasmids, or plasmid genes into chromosomes. Also rates of integration of chromosomal genes and integrons into plasmids or integrative-conjugative elements, transposons into plasmids, transposons inside other transposons, and transposons into chromosomes have to be taken into consideration. At a different hierarchical level, transmission of bacterial cells into secondary hosts (R_0), clonal integration of pathogens into host microbiota, and extent of clonal coexistence in the hosts must also be analysed (85). The 'integration' of particular genetic elements, and the efficient expression of resistance genes in recipient bacterial cells (49), requires that the overall regulatory and metabolic circuits of the recipient are compatible with the newly incoming elements (21). In fact the 'lack of integration' reflects increased fitness costs imposed by the unfit combinations of evolutionary units (see above).

The fourth main parameter is *replication rates*. The eco-evolutionary combination resulting from donor–recipient contacts, effective transfer, and integration (maintenance) of elements involved in antibiotic resistance in the recipient is successful only if it can be associated with a critical density, resulting from the replicative efficiency of each evolutionary unit (from genes to microbiotas) and their combinations. Therefore, replication rates correspond to all evolutionary units involved in antibiotic resistance. Of course this parameter refers to growth rates of bacterial clones *in vitro*, but also growth rates of bacterial clones in the host, and the maximum carrying capacity in the host (maximal population size) should be taken into consideration. Gene replication (beyond genome replication) also considers gene amplification and gene conversion (86). Indeed, plasmid, transposon, and integron replication (copy number) influences resistance gene replication rates. Note that many of these structures, as plasmids, are highly modular ones. The possibility that a specific resistance gene associates with a frequent module (as transposons) might increase its propagation.

The fifth main parameter refers to *diversification rates*. A key process in biology of antibiotic resistance is the ability of the different units involved to evolve (evolvability). Diversification offers the necessary material for natural selection, and finally to adaptation and evolution. Among the values that should compose the diversification parameter, we should consider measuring bacterial population mutation frequency

or, better, mutation rate per gene per cell and generation, inducible mutation rates, recombination rates, rates of clonal diversification within clonal complexes, clonal diversification into defined ecotypes, and migration (87). Similar measurements can be applied to lower units, such as diversification in particular insertion sequences, transposons, plasmids, and integrative-conjugative elements, as well as supracellular units, such as the overall microbiota present in a given ecosystem.

The sixth parameter refers to *selection rates*. All previous parameters determine the probability of the presence of particular multi-hierarchical constructions of variable complexity that are hooked by natural selection. Selection rates obviously depend both on selectable objects and constructions, and on selective forces acting upon them. In the case of antibiotic resistance we need to study the selection rates of different antibiotics at different concentrations for each mechanism of antibiotic resistance in the different cellular contexts, including different species and particular clones. Of particular interest is the determination of selection rates at subinhibitory concentrations of different antibiotics and, in general, at different times of exposure at particular concentrations (related with PK/PD selection) (5,88–92). Indeed, the determination of selection rates also should consider other parameters such as different growth conditions and ecosystems such as soil, sewage, or water bodies; different cell densities, including biofilm mode of growth; different mutation rates; combinations of antibiotics, or combinations of determinants of resistance to several antibiotics; or even selection rates in the presence of antibiotic-detoxifying organisms (93). Selection rates might also be relevant for antiseptics, biocides, and heavy metals for which a role on cross-selection of antibiotic resistance has been suggested (94–99).

Antibiotic resistance in a multi-parametric space: a chain reaction

Microbiologists, infectious disease specialists, epidemiologists, and public health officers have until now been 'well behind the facts' in antibiotic resistance. On the one hand, we are sometimes over-alarmed by the emergence of events of antibiotic resistance that never evolve beyond a first burst. On the other hand, the speed with which certain antibiotic resistances spread over the entire world (mimicking a chain reaction) are difficult to explain considering simplistic explanations, such as local patterns of antibiotic use. In fact a chain-reaction dynamics might well occur if the sixth parameter (selection rates) is feeding the first one (contact rates), and this one all others in a circular way. Thus, when the intensity of selection is high, we can expect a cascade effect of increases in contacts, transfer, integration, replication, diversification, and selection rates. Without considering the multi-parametric space analysed above, it will be hard to imagine the reasons (certainly not simple reasons) explaining the extremely rapid worldwide spread of antibiotic resistance genes among a huge diversity of clonal and species background, and the surprising diversity of the vectors (plasmids, transposons, integrons) harbouring resistance genes. In summary, the purpose of this article has been to show that the emergence and spreading dynamics of antibiotic resistance can perhaps only be understood in a multi-parametric space. We are conscious of the complexity of defining such a space, but a definition of that kind implies the possibility of focused interventions. Probably we should progress in the coming years in applying novel technologies, and developing novel rapid procedures to measure at least a critical number of the parameters that are mentioned above.

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