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Ecology of antimicrobial resistance: humans, animals, food and environment

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Summary. Antimicrobial resistance is a major health problem. After decades of research, numerous difficulties in tackling resistance have emerged, from the paucity of new antimicrobials to the inefficient contingency plans to reduce the use of antimicrobials; consequently, resistance to these drugs is out of control. Today we know that bacteria from the environment are often at the very origin of the acquired resistance determinants found in hospitals worldwide. Here we define the genetic components that flow from the environment to pathogenic bacteria and thereby confer a quantum increase in resistance levels, as resistance units (RU). Environmental bacteria as well as microbiomes from humans, animals, and food represent an infinite reservoir of RU, which are based on genes that have had, or not, a resistance function in their original bacterial hosts. This brief review presents our current knowledge of antimicrobial resistance and its consequences, with special focus on the importance of an ecologic perspective of antimicrobial resistance. This discipline encompasses the study of the relationships of entities and events in the framework of curing and preventing disease, a definition that takes into account both microbial ecology and antimicrobial resistance. Understanding the flux of RU throughout the diverse ecosystems is crucial to assess, prevent and eventually predict emerging scaffolds before they colonize health institutions. Collaborative horizontal research scenarios should be envisaged and involve all actors working with humans, animals, food and the environment. [**Int Microbiol** 2012; 15(3):101-109]

Keywords: ecology of antimicrobial resistance · eco-evo drugs · antibiotics · resistance units · EU antimicrobial policy · public health

Introduction: Learning is not compulsory... neither is survival

The problem of antimicrobial resistance is multifactorial, as we will discuss here. In addition, current actions to prevent antimicrobial resistance are not sufficient, and the problem is worsening. Among the new approaches to hindering the spread of antimicrobial resistance units (RU) are the use of eco-evo drugs [7,51] such as COINs (conjugation inhibitors, which prevent the transfer of resistance plasmids) [30]; blocking bacterial social behavior and communication through interference with quorum sensing mechanisms [19,65,67]; the old/new idea of using bacteriophages to specifically target prevalent clones/species; fighting biofilms [16,24]; and other, complementary approaches (for a review, see [12]).

The ecological approach does not focus on the development of new molecules, but rather on a novel way of thinking. The problem of antimicrobial resistance is not an individual one. Each antimicrobial molecule is a potential selection determinant. Antimicrobial molecules are not restricted

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to the treated patients, either animals or humans, but rather circulate throughout the whole ecosystem. Whether they are clinically used, (e.g., hospitals, animals, wastewater) or not (e.g., producers), antibiotics elicit selection and the spread of RU [39]. We thus face an ecological problem, with ecology defined in this context as the study of the relations that living organisms have with each other and with their natural environment. The ecology of antimicrobial resistance is in line with conceptual terms in the framework of curing and preventing disease. This relationship is of utmost conceptual interest and operational relevance as the causal relationships, and thus the ecology of antimicrobial resistance, properly embrace both ecological and evolutionary theories. This discipline approaches translational research and systems biology as unifying entities, in which the flux of resistance determinants is studied, from their origin to our hospitals.

We are now in an antimicrobial bubble, analogous to the real estate bubble that burst in many countries around 2010. As with all types of economic bubbles, they are generally identified retrospectively, after they have peaked and burst. We believe that the burst of the antimicrobial bubble is near, given the generalized passiveness of society to alarming discoveries of emerging and spreading pan-resistant pathogens that we must set an end to. Common multidisciplinary efforts including research on humans, animals, food and environment are not a possibility, but a need and a lesson we have to learn… if the goal of humanity is its own survival.

A brief historical perspective

After Alexander Fleming's discovery of penicillin in 1928, the use of antibiotics meant a giant leap forward in medicine. The availability of antibiotic chemotherapy deeply changed society by prolonging life expectancy and by allowing rapid population growth through both a reduction in infant mortality and the ability to treat common infectious diseases such as tuberculosis and other lung diseases, leprosy, bacterial meningitis, and sexually transmitted diseases. Infectious diseases were the major cause of hospitalization in the pre-antibiotic era. Unfortunately, this is still the case in developing countries. The introduction of penicillin G (in 1941) and streptomycin reduced mortality by 30 % in 1947 compared to 1938, and life expectancy world-wide extended seven years, from 49 to 56 years, between 1955 and 1970 [11]. As an example, in Britain, infant mortality due to congenital syphilis dropped from 1.5 in 1910 to 0.01 cases per 1000 in 1954 due to the introduction of penicillin G. During the decades following the discovery of penicillin, many new molecules with bactericidal activity were found in nature (Fig. 1), and some were even fully synthesized *in vivo*. Thus, within a few years, many life-threatening diseases became curable. It was believed, at least for some time, that infectious diseases would no longer be a threat to humankind. Hence, antibiotics were extensively used in human and veterinary medicine for the treatment of many infectious diseases. The observation that animal's daily weight gain increased with the use of low doses of antibiotics led to the massive use of these molecules also as growth promoters [34]. Unfortunately, soon after antibiotics where extensively used, antibiotic-resistant (AR) bacteria emerged, proving previous assumptions on the end of the infectious disease threat to be wrong. Nevertheless, it was believed that bacterial mutation frequencies were low enough to allow antimicrobial multidrug resistance to be largely ignored and measures aiming to reduce AR were accordingly not envisaged.

Antimicrobial resistance today

Antibiotics remain of the utmost importance, as many of the medical interventions, from routine wound healing to cutting-edge techniques such as organ transplantation or cancer and AIDS chemotherapy, rely on the efficacy of these molecules. Unfortunately, the overall picture of infectious diseases and antimicrobial chemotherapy is nowadays much more complicated and dramatically less optimistic, with multidrug resistant bacteria posing a real threat and thus a source of major concern. In fact, bacterial resistance to antibiotics is, currently, directly responsible for 15 times as many deaths as AIDS every year in Europe. This increasingly perturbing phenomenon has recently become one of the top six health topics addressed by the European Centre for Disease Prevention and Control.

Bacteria have shown an enormous evolvability towards resistance. Today, genetic mechanisms of the acquisition and transfer of antimicrobial resistance are well known. With the massive use of antibiotics in human and veterinary medicine, the advantageous effect of antimicrobial resistance determinants has soared. Due to selective pressure, dependent on the extent of antibiotics usage, most current European health policies mandate the responsible consumption of these drugs. Consequently, the use of these compounds as growth promoters in animals, a common practice in Europe until very recently, is now forbidden in the European Union (EU). Several studies have shown the impact of feeding antibiotics

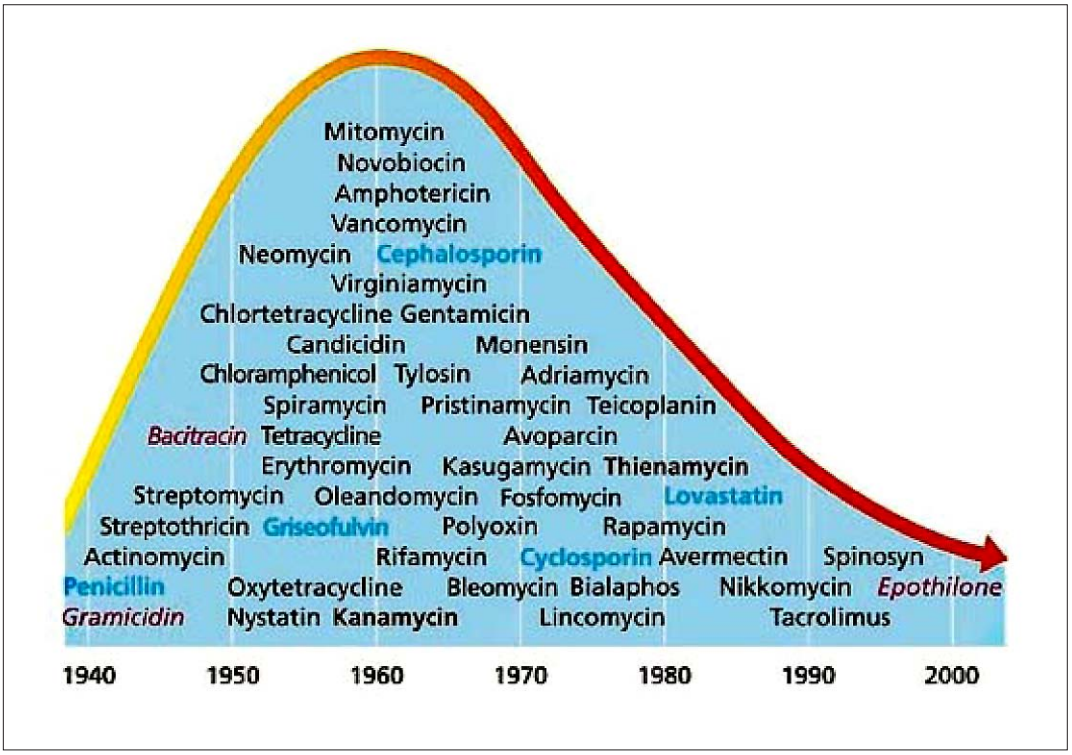

Fig. 1. Chronology of the discovery of new antibiotic classes. The dramatic decrease in the development of new molecules is a worrisome phenomenon. Normal type: actinomycete bacteria products. Blue: fungal products. Red: non-actinomycete bacteria products. (From [38], by permission of Oxford University Press, Inc.)

as growth promoters to food animals on resistance to clinically relevant antibiotics. The best-known example is the use, in poultry and pig production, of avoparcin, a member of the glycopeptides family and similar to the human antibiotic vancomycin. After avoparcin was banned for use as a growth promoter in Denmark in May 1995, the number of vancomycin-resistant enterococci (VRE) in both animals and humans decreased [5]. The EU decided, then, to ban its use in the rest of the Member States in April 1997. The same phenomenon was observed in several other countries including Italy and the Netherlands [43,52,63]. The Danish experience remains an important example of good management of antimicrobial policy in a country [1,2]. Larger countries with somewhat more complex structures must follow the same path, sooner or later.

Newer policies regarding antimicrobial use aim to increase the awareness of the population on the need to preserve the efficacy of antimicrobials. A clear example of these policies is the European Antibiotic Awareness Day, marked annually on the 18th of November. In accordance, drug resistance was the topic chosen for World Health Day in 2011 (7 April 2011) by the World Health Organization (WHO).

In contrast to the increasing levels of resistance, the discovery of new molecules has been in constant decline for the last several decades and there are no encouraging perspectives in the drug discovery field (Fig. 1). Unfortunately, containment seems to be the major asset in combating resistance. Knowledge on the molecular basis of antimicrobial resistance is crucial to avoid the acquisition and to hinder the spread of resistance determinants. We now know that bacteria efficiently recruit and mobilize AR genes by means of genetic structures that exceed by far the initial expectations concerning their genetic plasticity. Especially unexpected was the case of integrons, genetic platforms that can capture, stockpile, rearrange and differentially express genes, in subtle coordination with bacterial stress [36,50]. Integrons, transposons and plasmids render gene mobilization and capture so frequent that basic concepts and definitions are being revisited. For instance, nowadays it is considered that any given species has a core genome, with a stable content, and an accessory genome (in some cases comprising a large portion of the total DNA content), in which genetic modifications can be frequent. Consequently, we now talk about the pan-genome of a species, defined as the totality of genes that

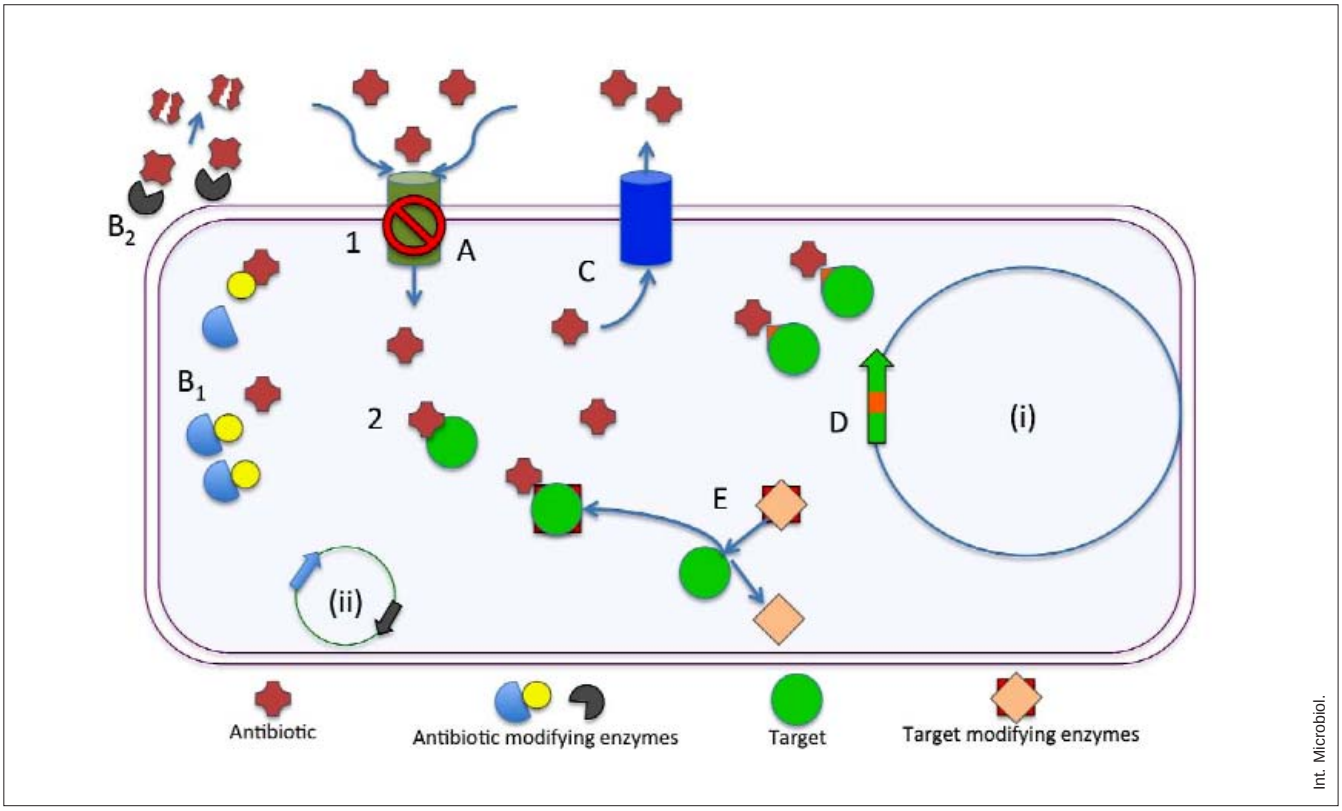

Fig. 2. Schematic representation of the most common resistance mechanisms. 1: Entrance of the antibiotic through porins. 2: Binding to the target. A: Loss of porins. B: Antibiotic inactivating enzymes by modification (B1) or by hydrolysis (B2). C: Enhanced efflux. D: Pre-transcriptional modification of the target. E: Post-translational modification of the target. (i) Bacterial chromosome bearing the gene encoding the target. (ii) Resistance plasmid bearing genes encoding for modifying enzymes.

have been found in members of a given species. In the pangenomes of clinically important bacteria, antimicrobial resistance determinants are now prevalent.

The mechanisms

Mechanisms of antimicrobial resistance can be grouped in seven classes:

1. Drug inactivation. Bacteria produce enzymes that can modify the drug, rendering it inactive or less active at therapeutic concentrations. These enzymes are efficient and, therefore, common among clinical isolates. The chemical modification induced in the antibiotic molecule can vary. Some enzymes bind radicals to the antibiotic, as is the case of chloramphenicol acetyl-transferases (CAT), aminoglycoside adenylases and phosphotransferases; others, like β-lactamases, hydrolyze the antibiotic molecule. Although the genes coding for those enzymes are frequently harbored in plasmids, it has been speculated that these genes originate from the chromosomes of yet unknown bacteria, in which they probably accomplish physiological functions. Once mobilized and overexpressed, these genes become resistance determinants. Supportive evidence for this hypothesis is the β-lactamase encoded by *ampC*, a resistance gene common in clinical isolates and identified in the chromosomes of two strains of β-lactam-susceptible *Citrobacter freundii* isolated during the 1920s [8], i.e. long before the clinical use of antibiotics.

2. Target modification. Antibiotic activity is dependent on the high affinity these molecules have for structures that are necessary for bacterial metabolism and integrity. Some of the bacterial targets of antibiotics can undergo structural modifications and still accomplish their function. When these modifications result in a decrease in the affinity of the drug, this process becomes a resistance mechanism. Some of these modifications are pre-transcriptional, with mutations in the genes encoding the targets that will ultimately give rise to

amino acid modifications in the protein. Others are post-translational, when modifications in the already-synthesized target are at the basis of the decrease in affinity. A good example of pre-transcriptional modification is the main mechanism of resistance against fluoroquinolones (FQs), in which mutations in *gyrA* and *parC* (genes coding for a subunit of the FQ targets) entrain amino acid substitutions responsible for a decrease in the affinity of the antibiotic for the target, while preserving the protein's activity [28]. A more recent example of post-translational modification of the target is the group of 16S rRNA methylases, discussed below. These enzymes confer high levels of resistance to aminoglycosides by simply adding a methyl group to the ribosome [32,37]. The resulting interference with antibiotic binding is dramatic, as resistance can increase up to 100-fold.

3. Reduction in the intracellular concentration of the antibiotic. Antibiotic molecules must generally reach the cytoplasm to exert their function, because many targets of antibiotics are intracellular. Thus, preventing the accumulation of these compounds in the cytoplasm leads to reduced susceptibility. This can be mediated either by the loss of porins in the cell wall, therefore limiting the entrance of molecules into the cell, or by the enhanced efflux of molecules. Efflux is mediated by proteins that span the wall and act as pumps, extruding compounds from the cytoplasm to the extracellular environment. Overexpression of these molecules or changes in their substrate specificity can lead to antimicrobial resistance [29,33,54]. This mechanism is especially worrisome due to the ubiquitous presence of efflux pumps in living organisms, and because efflux pumps often show a multidrug spectrum of substrates [45].

4. Reduced target expression. A decrease in the availability of the topoisomerase IV has been observed to be at the origin of low-level FQ resistance in *Staphylococcus aureus* [40]. Antibiotic targets are, by definition, molecules that are essential for bacterial survival. Thus, this is an example of plasticity in the rise of resistance. Another example of extreme reduction in target expression is that of glycopeptide resistance mediated by the *van* family of operons. These resistance operons allow abolishing the synthesis of glycopeptide targets (peptidoglycan precursors ending in D-Ala), and their replacement for D-Lac or D-Ser-ending variants showing a decreased affinity for the antibiotics [20].

5. Target protection. This phenomenon is observed for Qnr proteins, which mimic DNA structure and thus interfere allosterically with the binding of FQs to their targets, resulting in rising FQ resistance levels [62]. These determinants are currently spreading among clinically relevant enterobacteria but they have also been found in gram-positive bacteria such as enterococci [4].

6. Antibiotic sequestering. In this resistance mechanism, the molecules are not inactivated but instead are simply hampered or trapped in other structures. Examples of this mechanism are bleomycin resistance [27] and the cell wall thickening observed in glycopeptide-intermediate *Staphylococcus aureus* (GISA) [21].

7. Defective antimicrobial activation. Some antibiotics are pro-drugs and need to be activated by bacterial or host enzymes in order to become bactericidal. Resistance to these agents can therefore be mediated by the loss of the corresponding enzyme, its function, or its affinity for the antibiotic [58].

Antimicrobial resistance is ancient

As mentioned before, antimicrobial resistance is not a novel phenomenon. Antimicrobial resistance genes dating back to 30,000 years ago have been found in highly diverse collections of genes encoding resistance to β-lactam, tetracycline and glycopeptide antibiotics [22]. The origin of these genes are often antibiotic producers or bacteria cohabiting with producers, although not always, as discussed later. Most antimicrobials used to date come from bacteria or fungi inhabiting different ecological niches in nature [46]. In the case of bacteria, the microorganisms have to avoid their own suicide as a consequence of producing high amounts of antibiotics. These resistance determinants have been selected throughout thousands of generations and are thus very effective in their hosts. Resistance elements can then be transferred to bacteria residing in the same niche through horizontal gene transfer (HGT) events, mainly based on conjugation, transformation and transduction. In new genetic backgrounds, these genes are not always successful; rather, selection depends on several factors, which can be explained with the four Ps [7]: (i) **P**enetration refers to the ability of a genetic element from a particular system to enter and be present in other systems. Examples are RU that confer high-level resistance to antibiotics in antibiotic producers, which ultimately provide the same function in pathogenic bacteria. (ii) **P**romiscuity is the ability to exchange genetic sequences with other members of the ecosystem. (iii) **P**lasticity is the tolerated variability in the

genetic sequences, ranging from changes in the sequence of nucleotides in a gene to changes in the order of genes in a genome (synteny), in the modular structure of genetic regions (modularity), or in the modulation of their expression. (iv) **P**ersistence is the ability of each resistance unit to "construct a niche" in a specific context so that permanent links with its surrounding environment (including the genetic context) are established, allowing long-term coexistence or fixation of the sequence or element in the system.

Thus, effective antimicrobial resistance genes from antibiotic producers are not *per se* efficient resistance determinants in pathogenic bacteria. However, the reverse can be stated: all acquired resistance determinants in our hospitals have an origin in the environment.

Ecological dimensions

Ecology is classically defined as the study of the relations that living organisms have to one another and to their physical surroundings. Expressed more abstractly, ecology can be seen as the study of the relations and interactions of any set of actors in a given framework, both among themselves and with the framework as a whole. Antimicrobial resistance has not one, but several ecological dimensions.

The macroscopic dimension. Antimicrobial resistance in hospitals and in the community is a multifactorial problem. From a public health point of view, the concern that bacteria will become resistant during antibiotic treatment is negligible compared to the impact of the dissemination of resistant bacteria in the environment. Our current lifestyle allows for are extremely frequent and essentially borderless interactions with other humans, with resistant microbes being transmitted and transported between virtually any two points of the globe. As mentioned above, in this scenario animals can have a major impact on the origin of resistant clones as well as acting as reservoirs thereof. Transmission from animals to humans can occur through direct contact with living animals, but also through food obtained from animals colonized with resistant bacteria. Food-borne resistance also allows, through imports and exports, for an international circulation of resistance genes. Finally, some abiotic factors in this framework are also important in preventing the emergence of resistance. Antibiotics are currently being accumulated in the environment and some molecules such as FQs have long half-lives. Indeed, the delivery of antibiotics to the environment has to be minimized in order to decrease selective pressure and to limit the occurrence of new mechanisms of resistance [3]. Measures aiming to do so can range from the obvious need to control disposal of the molecules during their industrial production, to the lesser one of preventing waste materials from patients undergoing chemotherapy from reaching the environment.

The microscopic dimension. Antimicrobial resistant clones live together with many other bacteria in extremely complex habitats, such as the gut, skin, and mucosae of animals and humans, or natural environments. In these settings, communication, cooperation and competition take place continuously among billions of members belonging to hundreds of different species [35,53]. The microscopic dimension is currently being redefined through the use of massive sequencing technology, which has revealed an enormous level of complexity. The number of ecosystems is virtually infinite, and bacteria are not restricted to a given niche, but can move from one to another. This circulation of bacteria between humans and the environment provides a link between the environmental genetic pool and the microbiome in such a way that selective pressure in either system will eventually have an impact on public health.

DNA (including resistance markers) can be acquired from the environment through transformation, or from other members of the community through conjugation or transduction. Consequently, the success of resistance versus susceptibility (and therefore the probability of dissemination of resistant clones) depends mainly on one factor: fitness. In a given scenario, fitter bacteria overgrow other populations and prevail [66]. It is generally assumed that the acquisition of resistance confers a fitness advantage during antibiotic treatment but that it entails a fitness cost in the absence of antibiotics [60]. Hence, resistant clones should be cleared from a population if the antibiotic is discontinued [44,61]. Nevertheless, several phenomena, such as the accumulation of compensatory mutations in other loci, can counteract this fitness loss [10]. Also, the co-occurrence of two or more resistant phenotypes in a cell (multiresistance) can enable a co-selection phenomenon. In a clinical environment with recurrent antibiotic treatments, co-selection can compensate the fitness cost of a gene in the absence of the antibiotic to which it confers resistance. The selective advantage of the resistant strain harboring these mechanisms enables it to proliferate and spread, ultimately overgrowing the susceptible strains and stabilizing the determinants within the population. In addition, several

mechanisms are so efficient that they induce little if any fitness cost, proving that the acquisition of resistance determinants can be a win-win situation [31]. Of all the potential elements that are found in environmental and human resistomes, only the fittest prevail in hospitals.

The molecular dimension. Horizontal gene transfer is an evolutionary highway for bacteria. Therefore, the success of an antimicrobial resistance determinant at the molecular level depends mainly on its mobilization capacities. Plasmids currently play a major role in the mobilization of resistance genes between cells. Intrinsic features of plasmids, such as their host range, their incompatibility group [15], or whether they are conjugative or can be mobilized [59], directly influence the spread of the resistance marker they harbor. Other factors, such as the linking of different resistance genes within a plasmid, also influence the success of a given resistance gene as it allows for an undesired co-selection phenomenon that increases its persistence [14]. The transfer of resistance markers between replicons can be accomplished by transposons, allowing for the circulation of resistance genes among plasmids or between plasmids and chromosomes. Also, gene recruitment platforms such as integrons help to acquire new genes and are themselves associated with transposable elements. Nowadays, it is commonplace to find in hospitals resistant clones bearing several antimicrobial resistance genes within a class 1 integron, associated with a Tn402 transposon, embedded in a Tn21 transposon [13] and borne by a conjugative plasmid. Furthermore, upon antibiotic treatment, the bacterial SOS-response (triggered by many different antibiotics) [6] activates an integrase that reshuffles the integron content until it acquires a fitter conformation [9].

Altogether it is clear that the ecology of antimicrobial resistance has no political borders and that the need for a common international effort involving policy makers, researchers and health professionals from all fields is critically urgent.

Antimicrobials: the driving force from the environment to our hospitals

Today we know that antimicrobial resistance genes in hospitals have an origin in nature. This does not mean that the role of the RU was always related to antimicrobial resistance [47]. For instance, resistance genes like the FQ resistance determinants *qnr* have been found in environmental bacteria such as

Fig. 3. Resistance through 16S rRNA methylation. A single methyl group at position N7 of nucelotide G1405 in the 16S rRNA (red circle) is enough to hinder binding of 4–6-disubstituted aminoglycosides to the ribosome, conferring high-level resistance to these drugs. This resistance mechanism is used by aminoglycoside producers and pathogenic bacteria.

Aeromonas spp., residing in geographically unrelated water bodies [17,55]. Qnr proteins confer resistance to FQs, although the latter are fully synthetic antimicrobial molecules that were not present in nature before the mid 1950s. These resistance genes are found in the chromosome of gram-positive and gram-negative bacteria [41,42,57], with marine bacteria thought to be the origin of clinically relevant alleles [18]. As late as 1998, Martínez-Martinez and co-workers identified the first transferable FQ-resistance determinant [49], which is now very prevalent in pathogenic bacteria worldwide. Although the role of *qnr* genes in nature is not related to antimicrobial resistance, we know that these genes have been present in environmental bacteria for thousands, if not millions of years. Selection through the use of FQs has been the driving force that has brought these elements to hospitals.

In some other cases, the function of the RU in nature is

retained by its diverse bacterial hosts in hospitals. This is the case of the 16S rRNA methyltransferases, which confer high-level aminoglycoside resistance. These enzymes specifically methylate a single nucleotide of the 16S rRNA ribosome, G1405. With a methyl group in this position, 4–6-disubstituted aminoglycosides such as tobramycin, amikacin or gentamicin, the clinically most commonly used molecules of this family, are sterically hindered and thus unable to bind to their site of action, the aminoglycoside-binding pocket (Fig. 3).

Aminoglycoside-producing bacteria have developed methods to ensure their own protection. For example, to protect itself from the gentamicin it produces, *Micromonospora purpurea* adds a methyl-group to the G1405 position of its own ribosome, resulting in high-level resistance to the antibiotic. Enzymes of the same family, such as ArmA and RmtA-F have been identified mainly in enterobacteria [23,25,26,32, 64,68]. Some of these enzymes have been shown to likewise modify nucleotide G1405 by the addition of a methyl group, again conferring high-level resistance to aminoglycosides and hence rendering these antibiotics useless against bacteria that have acquired these genes. The gene encoding RmtC has further integrated into the chromosome of *Salmonella enterica* serovar Virchow [37] without affecting the fitness of the host bacterium. Thus, acquired genetic elements from nature can retain their function and encounter adaptive mutations that enable them to preserve their original environmental function in pathogenic bacteria from hospitals [56]. Furthermore, these genes have been identified in animal isolates as well as in isolates from food, proving, again, that the path from the environment to the hospital involves not only humans and environmental bacteria but also animals and food products.

Antimicrobial resistance is an increasingly dangerous phenomenon. Thus, efficient collaboration between all actors involved is crucial to identify, assess and predict RU before they colonize our hospitals [48]. In this sense, the ecology of antimicrobial resistance is an emerging discipline that is contributing new models and solutions to combat this uncontrolled pandemic.

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SEM Biennial Prize

The Spanish Society for Microbiology (SEM) Biennial Prize dates back to 1983, when the SEM decided that a lecture should be given by a young researcher at each SEM National Congress. The nominees are selected from among the SEM membership; they must be under 40 years of age, and carrying out research of excellence in a field of microbiology. The following researchers have been awarded the SEM Biennial Prize (the centers indicated are those where the scientists worked when they received the prize).

• First: **Juan Ortín**. Center for Molecular Biology (CBM), CSIC-Autonomous University of Madrid (10th SEM National Congress, Valencia, 1985)

• Second: **Enrique Herrero**. Department of Microbiology, University of Valencia (11th SEM National Congress, Gijón, 1987)

• Third: **Ernesto García López**. Biological Research Center (CIB), CSIC, Madrid (12th SEM National Congress, Pamplona, 1989)

• Fourth: **Antonio Ventosa**. Department of Microbiology, University of Sevilla (13th SEM National Congress, Salamanca, 1991)

• Fifth: **Alicia Estévez Toranzo**. Department of Microbiology, University of Santiago de Compostela (14th SEM National Congress, Zaragoza, 1993)

• Sixth: **Sergio Moreno**, Department of Microbiology, University of Salamanca (15th SEM National Congress, Madrid, 1995)

• Seventh: **Daniel Ramón Vidal**. Department of Biotechnology, Institute for Agrochemistry and Food Technology (IATA), CSIC, Valencia (16th SEM National Congress, Barcelona, 1997). See *Microbiología SEM* 1997; 13(4):405-412

• Eighth: **José Antonio Vázquez Boland**. Department of Animal Pathology, Complutense University of Madrid (17th SEM National Congress, Granada, 1999). See special issue on Microbial Patogenesis in INTERNATIONAL MICROBIOLOGY 1999; 2(3):131-198

• Ninth: **Jesús L. Romalde**. Departament of Microbiology and Parasitology, University of Santiago de Compostela (18th SEM National Congress, Alicante, 2001). See INTERNATIONAL MICROBIOLOGY 2002; 5(1):3-9

• Tenth: **Eduardo Díaz**. Biological Research Center (CIB), CSIC, Madrid (19th SEM National Congress, Santiago de Compostela, 2003). See INTERNATIONAL MICROBIOLOGY 2004; 7(3):171-178

• Eleventh: **Iñigo Lasa**. Institute of Agrobiotechnology and Department of Agrarian Production, Public University of Navarra-CSIC, Pamplona (20th SEM National Congress, Cáceres, 2005). See INTERNATIONAL MICROBIOLOGY 2006; 9(1):21-28

• Twelfth: **Luis A. Fernández Herrero**. National Center for Biotechnology, CSIC-Autonomous University of Madrid (21st SEM National Congress, Sevilla, 2007)

• Thirteenth: **Alejandro Mira**. Center for Advanced Research in Public Health (CSISP), Valencia (22nd SEM National Congress, Almería, 2009). See INTERNATIONAL MICROBIOLOGY 2010; 13(2):45-57

• Fourteenth: **Bruno González-Zorn**. Department of Animal Health, Faculty of Veterinary, Complutense University of Madrid (23rd SEM National Congress, Salamanca, 2011). See this issue, pp. 101-109