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When Humans Met Superbugs: Strategies to Tackle Bacterial Resistances to Antibiotics

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Abstract: Bacterial resistance to antibiotics poses enormous health and economic burdens to our society, and it is of the essence to explore old and new ways to deal with these problems. Here we review the current status of multi-resistance genes and how they spread among bacteria. We discuss strategies to deal with resistant bacteria, namely the search for new targets and the use of inhibitors of protein-protein interactions, fragment-based methods, or modified antisense RNAs. Finally, we discuss integrated approaches that consider bacterial populations and their niches, as well as the role of global regulators that activate and/or repress the expression of multiple genes in fluctuating environments and, therefore, enable resistant bacteria to colonize new niches. Understanding how the global regulatory circuits work is, probably, the best way to tackle bacterial resistance.

Keywords: Antibiotic resistance; superbugs; tensegrity; bacterial colonization; niches.

Abbreviations

AMR (Antimicrobial Resistance), WHO (World Health Organization), CDC (Centers for Disease Control and Prevention), HGT (Horizontal Gene Transfer), COINs (Conjugation inhibitors), MGEs (Mobile Genetic Elements), ICEs (Integrative and Conjugative Elements), IMEs (Integrative and Mobilizable Elements), T4SS (Type IV Secretion Systems), uFAs (unsaturated fatty acids), TAs (Toxin-Antitoxins), i-PPIs (Inhibitors of Protein-Protein Interactions), HTS (High Throughput Screenings), asRNA (Antisense RNA), PNAs (Peptide-Nucleic Acids)

Introduction

Many millions of Euros are spent every year on research programs aimed to improve human and animal health, but up to now, very few of them have benefitted from follow-up studies that determine if the investments have actually made a difference to human welfare. There is an important drawback, though, and it is that development of new drugs is, at present, immensely expensive because of failures between Phase 2 and submission due to toxicity and efficacy. Conversely, not enough funds are devoted to the prevention of drug resistance in microorganisms and to the development of new antimicrobials. This is a matter of utmost importance if we want to proceed with our well-being in developed countries and to develop strategies to increase the living standards of less favoured populations. The use, abuse, and misuse of antibiotics over the years have led to the selection of bacteria that are, at present, resistant to all known antibiotics (“superbugs”), for which we have scarce or no treatment. Bacterial resistance is especially dramatic in the hospital-acquired (nosocomial) infections, because any small surgery or anti-cancer treatment would be followed, in many cases, by severe bacterial infections. This, in turn, creates a feedback loop that leads to the selection of even more antimicrobial resistant bacteria (AMR) [1] . The previous admonitory terms of ”gloom scenario”, ”dark horizons”, and ”back to the pre-antibiotic era” are becoming obsolete [2], and we now have superbugs in our everyday health care. Further, we are running out of antibiotics as the World Health Organization (WHO) reports (http://www.who.int/news-room/detail/20-09-2017-the-world-is-running-out-of-antibiotics-who-report confirms), in spite of some optimistic studies on the discovery or the synthesis of possible new antibiotics [3, 4]. Predictions made by WHO estimate around 50 million human deaths by 2050 due to

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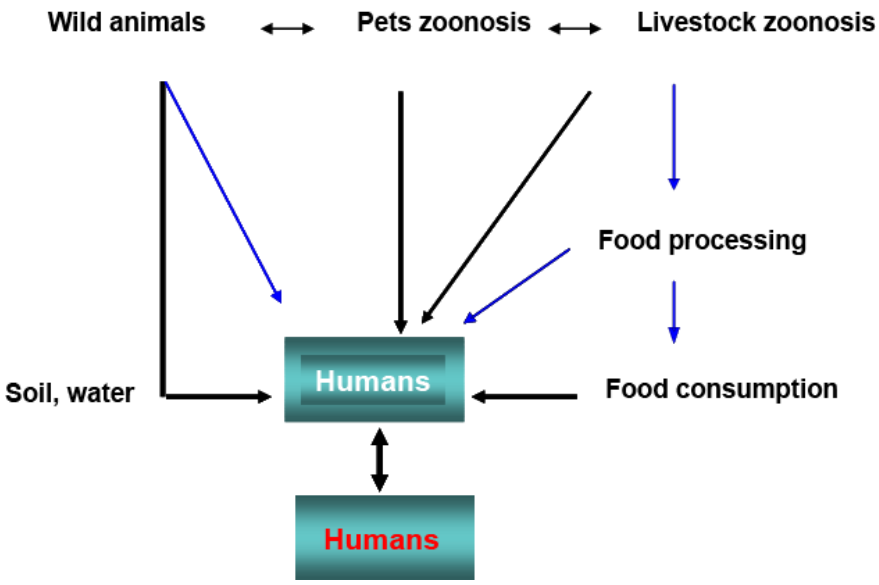

Figure 1: Main routes of transmission of antibiotic-resistant bacteria. Thick black arrows indicate the major routes, whereas thin blue arrows point to minor, but relevant ways of acquisition. A significant route of spreading the bacterial infections is from infected (red) to healthy (white) humans.

bacterial infections; the United Nations in their General Assembly in 2016 [5], and the G20 Health Ministers (http://www.g20.utoronto.ca/2017/170520-health-en.html) declared that the highest world health problem is the increase in the appearance of AMR bacteria. Indeed, the number of bacterial strains exhibiting increased resistance to antibiotics is expanding by the day, and there is a struggle to control the dissemination of AMR traits, as reflected by the releases of Health Agencies, like the WHO (http://www.who.int), and the Centers for Disease Control and Prevention (CDC) (https://www.cdc.gov/; http://www.ecdc.europa.eu), which provide periodical reports alerting on this urgent subject. In 2018, up to 154 countries have agreed to increase surveillance on the use and abuse of antibiotic consumption in humans and livestock. However, their reports show discrepancies and selling antibiotics without a prescription is still a wide practice in many countries. Infectious diseases caused by bacteria take a toll on human lives, in addition to a heavy economic burden on society. Furthermore, and notwithstanding some controversy [6], the use of antibiotics on farm animals adds a further risk to the occurrence of AMR bacteria. In this regard, a 2017 report by the European Union, commented by the European Medicines Agency, indicated a doubtless link between total antibiotic consumption and the occurrence of antibiotic resistance in both humans and farm animals (https://www.ema.europa.eu/news/eu-report-more-evidence-link-between-antibiotic-use-antibiotic-resistance), leading to a proposal of measures devoted to the responsible use of all classes of antibiotics to reduce the risk of "co-selection", that happens when the use of one type of antibiotic produces resistance to another (cross-resistances).

Here we will discuss why and where bacteria acquire their resistance, how the genes encoding AMR spread among bacterial populations, and which specific and global approaches have been or could be designed to combat such resistance.

Acquisition of AMR by Bacteria

Huge amounts of money are devoted every year to treat infections caused by pathogenic bacteria, and these expenses increase considerably in many cases because of the paid short sick-leaves caused by milder infections. Acquisition and spread of AMR is a serious matter that is further enhanced by globalization, including travellers, population migrations, and trade of agricultural and livestock products. This increasing mobility leads to broader dissemination rates of bacterial pathogens and spread of AMR. Additionally, bacterial infectious diseases are acquired from different sources, not only through human-human interactions but also from the environment and contact with other animals, including domestic pets, livestock, and wildlife (Figure 1). Moreover, the use of antibiotics in animals' feed or medical treatment results in the release of the drugs

through water or waste into the environment. There, an extreme selection allows mainly the survival of AMR bacteria, whereas the sensitive bacteria are eliminated. In addition, humans can be invaded by AMR bacteria (either pathogens or commensals) from these animal sources, and the genes responsible for the resistance can be transferred to the human microbiome through a number of different mechanisms (see below). Thus, feeding livestock antibiotics is a risk that has been evaluated, and the available figures are overwhelming. Antimicrobial sales for livestock could be obtained for 38 countries and estimated for 190 more. In 2013, the global consumption of all antimicrobials in livestock was calculated to be of 131,110 tons and is projected to reach 200,235 tons by 2030 [7]. This gives an idea of how much pressure is being exerted on bacteria, which leads to the selection of resistance in our environment and our livestock. We have to consider that nearly 50% of bacterial infectious diseases can be acquired by humans from animals, including pets, livestock, and wildlife, according to estimates published by the CDC Offices. Public health officials and scientists working in different fields are joining forces to understand the basic questions on how, when, where, and why bacterial pathogens are transferred to humans, in the hope of preventing epidemic and pandemic outbreaks. To perform this joint work, it is important to increase our efforts in the surveillance programmes that aim to detect early outbreaks and respond to them quickly, just in the place where these outbreaks are detected. Other important approaches involve the identification of factors that could affect the interspecies pathogen transfers, such as the immune response of infected animals, or the pathways of human exposure. Another interesting, but more difficult, approach is to map the distribution of species that host zoonotic pathogens and to search for reservoirs of pathogens. Even with an understanding of how transmission of interspecies pathogens happen and maps of zoonosis distribution, precise predictions of outbreaks do not seem to be feasible at least at present.

Transfer of AMR Genes

Most of the genes responsible for resistance to antibiotics are transferred horizontally among bacteria by a process called horizontal gene transfer (HGT) [8]. There are three main mechanisms of HGT: natural genetic transformation, conjugal transfer, and bacteriophage transduction (Figure 2). ***Natural genetic transformation*** (Figure 2A) usually takes place when a portion of a given bacterial population receives environmental signals relayed by quorum sensing molecules, which triggers the expression of a set of operons involved in the uptake of free DNA (genetic competence). Competence is a bi-stable process in which bacterial DNA is released by lysis of a subpopulation of noncompetent cells through a process termed “cannibalism or fratricide”, which is induced by a fraction of competent cells [9]. HGT is mediated mostly through ***conjugal transfer*** mediated by mobile genetic elements (MGEs): plasmids, integrative and conjugative elements (ICEs), and integrative and mobilizable elements (IMEs) that usually carry one or more genes encoding resistance to antibiotics [10]. The mechanisms that set off the conjugative process are not well understood. In some bacterial species, a pheromone that agglutinates the cells is secreted by the donor cells; however, this does not happen in all cases [11]. Conjugation implies the participation of two partners: donor and recipient bacterial cells. The donors encode all the machinery needed for the DNA transfer process, whereas the recipient appears to have a passive role [11]; (Figure 2B). In general, it has been estimated that around half of the large plasmids (above 30 kilobase pairs) are conjugative and bacteria devote a large part of their genome to encode all the proteins needed for their plasmid transfer” by “encode all the proteins needed for their transfer [12]. Most of these genes are clustered in operons involved in the synthesis of: i) the initiator of transfer (relaxase) and its auxiliary proteins, ii) the proteins participating in the assembly of the conjugation pilus, and iii) the specialized machinery participating in the injection of plasmid DNA from donor to recipient cells, the type IV secretion system (T4SS) [13]. HGT mediated by bacterial viruses (bacteriophages) is a process termed ***bacteriophage transduction*** (Figure 2C). It takes place when, after phage infection and lysis of the bacterial cell, some of the viral particles encapsidate pieces of the bacterial DNA creating transductant particles. Upon further infection, the transductant particles inject bacterial DNA into next host bacterium, creating a recipient bacterium that will not be killed but, on the contrary, will acquire new genetic traits.

Strategies to Tackle Transmission of AMR

Many of the MGEs are shared among bacteria of different species, and constitute a common reservoir of DNA that can be up to 20% of the bacterial pangenome [8]. This shared pool of DNA, termed the mobilome [14], would

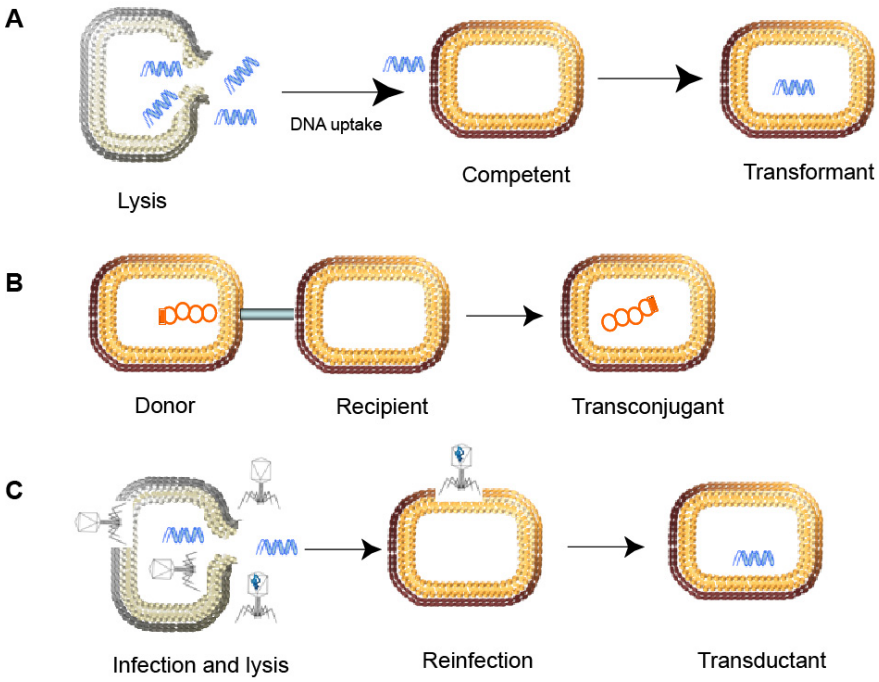

Figure 2: Main routes of horizontal gene transfer. **A.** Transformation takes place by a part of the bacterial population entering into a particular physiological state, mediated by a competence stimulating peptide, that makes them able to uptake 'naked' external DNA. Upon uptake, incoming DNA would recombine with the chromosome of the receiving cell, incorporating the new genes from the incoming DNA. **B.** Conjugation is mediated by cell-to-cell contacts in which the donor partner carries a plasmid (or any mobile genetic element) that encodes the genes needed for pilus assembly and the genes involved in the formation of the type IV secretion system protein complex channel. Through it, the plasmid DNA is transferred from the donor to the recipient cell. **C.** Transduction takes place when a phage infects a cell and is able to package pieces of chromosomal DNA rather than phage DNA. These phage particles are released after cell lysis and are able to infect other bacteria, injecting the chromosomal DNA that can be integrated into the chromosome, acquiring the new genetic traits.

account for the quick transmission of AMR genes even among evolutionary divergent bacteria. Consequently, the discovery of new antibiotics, albeit extremely important, does not seem to be the definitive solution to fighting infectious diseases [15]. Finding novel targets and strategies to deal with the spread of AMR should probably be the best approach we can envisage at present [2]. Thus, the methods to combat the emergence and spread of AMR genes should mainly rely on: i) drug discovery approaches, ii) combination and/or alternation of drugs, and iii) target bacterial functions essential for infection [16]. These methods can be merged with complementary approaches like decrease of antibiotic consumption, preservation of existing therapeutics, and general hygiene and containment. One interesting approach to deal with the transmission of AMR has been the discovery of conjugation inhibitors (COINs), first developed in the frame of a European consortium, which led to the finding that unsaturated fatty acids (uFAs) are effective COINs [17]. Among the proteins integrating the T4SS, there is one ATPase, generally termed VirB11, which seems to be the target of these uFAs [18]. The protein is able to bind palmitic acid one of the major components of the bacterial membrane; but the uFAs can replace palmitic acid and reduce the ATPase activity of VirB11, hindering conjugation [19]. Thus, it seems that a promising avenue to reduce the flux of AMR genes by HGT is becoming available, although so far this approach is limited in the number of plasmids and the bacteria assayed.

Not only is there no single antibiotic to cure all bacterial infections, but bacteria that have developed resistance to one antibiotic may display a greater sensitivity to a second one from a distinct structural class. This phenomenon known as ***collateral sensitivity*** has led to serious discussions in the design of novel drug discoveries [2, 20, 21]. Likewise, the combination of different therapies appears to be the most feasible approach, and alternative strategies to the classical treatments could be advisable. These strategies may include: i) search for chemical derivatives of the known antibiotics; ii) *pro tempore* use of

available antibiotics (antibiotic cycling or drug rotation), that is, to alternate one category of antibiotics with one or more different ones that exhibit comparable activity; this method, however, has been subjected to criticisms on the basis of lack of reliable clinical information [22]; iii) implementation of more biological approaches, like phage therapy [23], new vaccines, use of antimicrobial peptides, and exploitation of bacterial defence systems [24, 25]; iv) development of entirely novel classes of antibiotics; v) use of new small molecules as antivirulence or antipersisters drugs [26], and vi) targeting bacterial-encoded virulence factors [27].

Novel Targets and Novel Strategies to Fight Bacterial Infections

To succeed, at least partially, in the fight against bacterial infections, a number of different approaches focused on new targets or different strategies should be explored. We will briefly discuss some of those that have already attracted scientific attention and some of those that may be worthwhile to follow up on, even though they may be a bit speculative at the present stage of knowledge.

- ***Toxin-antitoxins as novel targets.*** The type II (proteic) toxin-antitoxins (TAs) systems are genetic modules present only in bacterial and archaeal genomes. In general, the modules are organized as bicistronic operons encoding two proteins: an unstable antitoxin and its cognate stable toxin. Both proteins generate a harmless protein-protein (T:A) complex that regulate their own synthesis. Furthermore, the antitoxin molecules usually wrap around and bury their cognate toxins to avoid the accessibility of the toxins to the outer surface of the protein-protein complex. Under environmental stress, especially nutritional stress or colonization of a new host, there is a drastic drop in the intracellular levels of the antitoxin, mostly due to degradation by endogenous proteases to which antitoxins are accessible. The T:A complex is then disrupted and the toxin is released, leading to either cell death or cell stasis [28]. Different toxins specifically affect essential cell processes, such as cell wall synthesis, DNA replication, ribosome assembly, or translation, with RNA cleavage being the most frequent mode of action [29, 30]. Based on: i) the ubiquity of TAs in bacteria, ii) the absence of these genetic modules in Eukaryotes, and iii) the possibility to trigger these intracellular poisons, some research has been devoted to TAs as potential targets for new antimicrobials [25, 31-36]. However, it has been found that the toxins of the T:A pairs can be useful as anti-virals [37, 38] or anti-tumoral agents [39] rather than as antibacterials, with the exception of two reports dealing with the possible drugability of the Epsilon:Zeta TA system (below). Reviews on the drugability of these TA genetic modules have been published, and their advantages and pitfalls have been analyzed in detail [2, 40-42].

- ***Interfering with the stability of the mobilome.*** Spread of AMR is largely due to genetic exchange mediated by the mobilome since resistance genes are present in plasmids, phages, IMEs, and ICEs. Thus, understanding the mobilome and how it moves among bacteria is essential to deal with AMR [14]. MGEs are mostly acquired by HGT, and employment of COINs could preclude horizontal transfer. However, in addition to being acquired, MGEs can also be lost, and this may constitute a yet unexplored way to cope with AMR by interfering with the mechanisms that ensure the conservation of the mobilome. In particular, inducing plasmid loss could be a strategy worth exploration. We can conceive this approach as follows: instead of interfering with plasmid conjugation that lasts from minutes to a few hours, or with the outcome of mutations that may occur within minutes, it could be worthwhile to interfere with plasmid-chromosome crosstalk that lasts a lifetime, as long as the plasmid is not lost. Then, the identification and employment of new molecules able to interfere with plasmid stability, alone or in combination with COINS, could lead to a substantial impulse in the battle against the spread of AMR.

- ***Targeting virulence traits.*** Additional approaches which may be complementary to the use of antibiotics envisage interfering with virulence traits rather than with biosynthetic or growth pathways. In this approach, the aim is not to kill the pathogenic bacteria, but rather to diminish their virulence, thus avoiding selection of resistances and allowing the combination of these strategies with the use of antibiotics [43, 44]. Alternatively, targeting genes involved in bacterial motility (e.g., flagella, fimbriae) or hindering the assembly of the proteins participating in these organelles could lead to a substantial reduction in the dissemination of the infection.

- ***Inhibition of secretion systems.*** In addition to the abovementioned T4SS, many bacterial genes are involved in secretion of macromolecules, especially those participating in the secretion of effector proteins involved in pathogenesis [45]. The search for inhibitors of these systems could be an effective approach to deal with virulence that would merit further detailed research.

The possible novel targets mentioned above are alternatives to the “classical” targets, which include the use of antibiotics to inhibit essential bacterial processes, such as protein or nucleic acid synthesis, cell

wall synthesis, or transcription or translation signals. Most of the "novel" strategies take into consideration structure-based approaches combined with computer-assisted docking of molecules with the aim of disrupting macromolecular interactions. We shall mention three relevant strategies that involve the use of: i) inhibitors of protein-protein interactions (i-PPIs), ii) small molecules or "fragments", and iii) antisense RNAs.

- Inhibitors of protein-protein interactions. Characterization of the surface contacts between two interacting proteins can be combined with new methods to perform docking of molecules and/or structure-based design. These approaches have permitted the identification of small molecules that could act as i-PPIs [46], such as the discovery of small inhibitors of the interactions between proteins ZipA and FtsZ. These proteins participate in the formation of the ring septum in the bacterial cell division ring known as divisome, and disrupting this interaction is an important target for drug discovery [47]. One of the approaches to using bacterial toxins from the T:A complexes as antibacterials has been to design and characterize short peptides that can dissociate the T:A interactions based on their binding interface [48]. This approach can be extended to other targets mentioned above, but it requires the knowledge of the high-resolution three-dimensional structure of the intervening proteins. Thus, the binding affinities of the candidate inhibitory peptides that could disrupt the protein-protein interface contacts could be determined. This is relevant when estimation of the dosage of the inhibitors is required. Calculation of the protein-protein interfaces is essential to develop new i-PPI molecules. In the case of the Epsilon:Zeta pair, whose structures have been solved [49, 50], there are two promising examples of synthetic peptides that disrupt the protein-protein interfaces. The first example involved the use of peptide libraries and the development of high throughput screening (HTS) approaches [51], whereas the second example was based on the definition of possible α-helix peptides that could disrupt the T:A pair interface [52]. These two findings have opened new avenues that merit in-depth exploration. Furthermore, the use of these approaches with other macromolecular assembly machineries (above) may extend our panoply of tools to combat infections. There are, however, two needs to be solved: i) the use of HTS techniques to identify hits that could act as i-PPIs, and ii) the development of standard protocols to optimize the analyses of hits to select proper leads, the so-called "hit-to-lead" approach.

- Fragments. The fragment drug discovery technology searches for small (<300 Da) molecules that provide at least 3 hydrogen-bond donors and acceptors. The goal of this approach is the discovery of low-molecular-mass and low-affinity molecules, so that later on they can be optimized into drug leads [53]. Alternatively to traditional HTS of peptide libraries, the fragment-based methods for drug discovery have emerged as an important approach to find new drugs within most pharmaceutical companies and many academic groups [54-56]. In addition, it has been shown that molecules identified by fragment-based screens have more drug-like properties than those identified by more conventional drug discovery techniques, which speaks in favour of the fragment approaches [55]. A scientific database in which a high number of fragments can be searched is presently available at http://gow.epsrc.ac.uk/NGBOViewGrant.aspx?GrantRef=EP/I037288/1.

- Antisense RNAs (asRNAs). Alternatives to the above strategies are under intense research, especially the employment of asRNAs to shut down translation. In this field, the more efficient approaches so far include the use of modified asRNAs coupled to oligodeoxynucleotides to silence essential genes at mRNA::DNA levels. However, these DNA:RNA hybrid molecules are easily degraded by intracellular nucleases. To avoid this inconvenience, variants of these molecules have been sought, especially molecules that are formed by peptide-nucleic acids (PNAs) [57]. The PNAs are analogues of nucleic acids in which the ribose/deoxyribose-phosphate backbone has been replaced by a flexible peptide polymer to which the bases are attached. Consequently, PNAs mimicry is a strategy to synthesize molecules that can pair to DNA or RNA, generating heteroduplexes that are resistant to intracellular degradation. Alternatives to these analogues have been developed by the use of glucose-DNA conjugates that have been fluorescently labelled to allow for intracellular detection, with promising results [58]. These approaches might have the drawback of the intracellular delivery of the compound [59], but alternatives have been found and clinical trials are in progress [60]. Some of the nucleic acid-based strategies have been compiled in a recent number of Nucleic Acids Research (https://academic.oup.com/nar/pages/nucleic_acids_therapeutics_collection).

Holistic approaches

Alternative points of view to the abovementioned ones have to be taken into account. For instance, Eco-Evo based proposals consider that AMR genes were widespread and prior to human intervention, since these traits can be traced back to the pre-antibiotic era, and genes conferring antibiotic resistance were detected in the microbiome of

environments dating back one million years [16]. Thus, the global Eco-Evo approaches consider that the final goal in fighting bacterial infections is not to kill all multiresistant microorganisms, but to prevent their emergence/evolution or to restore the antibiotic-susceptible populations that could struggle efficiently against the resistant ones. Some of these global strategies focus on interfering with the invasion, promiscuity, plasticity, and persistence of the genetic units responsible for AMR [16].

We should also consider that bacterial species live in communities within a given niche, where a delicate equilibrium must be reached to maintain the number of individuals and the number of species represented. Furthermore, bacterial communities are able to communicate with each other by means of sensors that report the fluctuations that take place in a changing environment. Within a global understanding of the pathogens, we have to be aware that colonization of a niche means that the invading bacterial population must compete with the already resident populations for the resources that this particular niche provides [61]. As a consequence, bacterial colonization of a new niche requires that many genes must be turned on or off depending upon the new environmental conditions. The new pattern of gene expression, which we define here as the bacterial ***nichome***, is mostly determined by global gene regulator proteins that act as repressors and/or activators. Understanding these complex regulatory networks is essential to effectively control bacterial infections. Colonization of a preferred niche and, later on, colonization of a new niche (e.g. *Streptococcus pneumoniae* moving from nasopharynx to lungs, *Escherichia coli* from the intestinal tract to the urine vesicle, or *Enterococcus faecalis*, from the human gut to the heart valves) will lead to profound changes in bacterial gene expression. In many cases, these radical changes in the expression of numerous genes occur not only at the transcriptional level but also at the post-transcriptional level. We can conclude that survival of a bacterial population in a new niche, or in a niche subjected to fluctuations, would depend on the activity of global regulators and, most importantly, on the connections established among regulatory networks.

Many bacterial species live in communities known as biofilms, which are defined as multicellular bacterial communities held together by an extracellular polysaccharide matrix [62] (Figure 3). Biofilms are glued together by a matrix that can adhere to various surfaces from human tissue [63, 64] to a stone in water [65], or to plant roots [66]; but in all cases there are, in addition to the sessile cells within the biofilm, other planktonic cells that may leave the biofilm to explore the possibility of colonizing other niches [67]. Bacteria growing in a biofilm are far less susceptible to antimicrobials than planktonic cells, because of the reduced accessibility of the drugs to the cells within the biofilm; thus infections in these cases are very difficult to cure [68]. Moreover, within a bacterial biofilm, HGT processes take place and although they seem to be limited to neighbouring cells, there is scarce information on the mechanisms underlying these processes [69]. In fact, experimental evidence points to a connection between biofilm formation and HGT. This connection would suggest the existence of yet unknown communication processes between cells within a biofilm [70]. Thus, communication between bacterial populations becomes an important factor to consider when dealing with selection of AMR bacteria or transfer of genes encoding resistance.

From a formal point of view, bacterial communities, especially those living within biofilms, can be considered as natural structures that organize themselves into tensegritic entities. Early in the 1960's, the architect R.B. Fuller, and soon after two artists (D.G. Emmerich in 1962 and K.D. Snelson in 1964), observed that many natural structures are kept together by a continuum of flexible elements, so that outside pressures are distributed evenly by a mechanical process termed "tensegrity" (from tension + integrity) [71]. Under tensegrity conditions, whole systems harbour a resiliency that helps maintaining their integrity and, ultimately, their interconnectivity [72] (Figure 4). Tensegrity systems do not only exist in physics, art, or architecture, they are also present in the biological world creating integrated connections, such as those present in the cell cytoskeleton [73, 74]. Resilience and interconnectivity are inherent to tensegrity systems, and the shapes they adopt are designed to support them [75]. Within a tensegritic system, all of the components are interconnected and the system constitutes a whole being, like a eukaryotic cell or a suggestive sculpture. Consequently, tensegrity in the biological systems implies the existence of networks that would influence gene expression, leading to cascades of protein signalling. If tensegrity implies interconnectivity within a single cell [73], this principle may apply to biomolecules other than DNA (which is *per se* a tensegrity system), like proteins that assemble-disassemble together (tubulins, for instance) or the components of the peptidoglycan [43]. These biomolecules will keep the functional interconnectivity among them and would also connect distant regions of a bacterial chromosome. Within bacterial populations the tensegrity principles would also apply, since biofilms, for instance, are an example of intercellular connectivity. We could then propose the

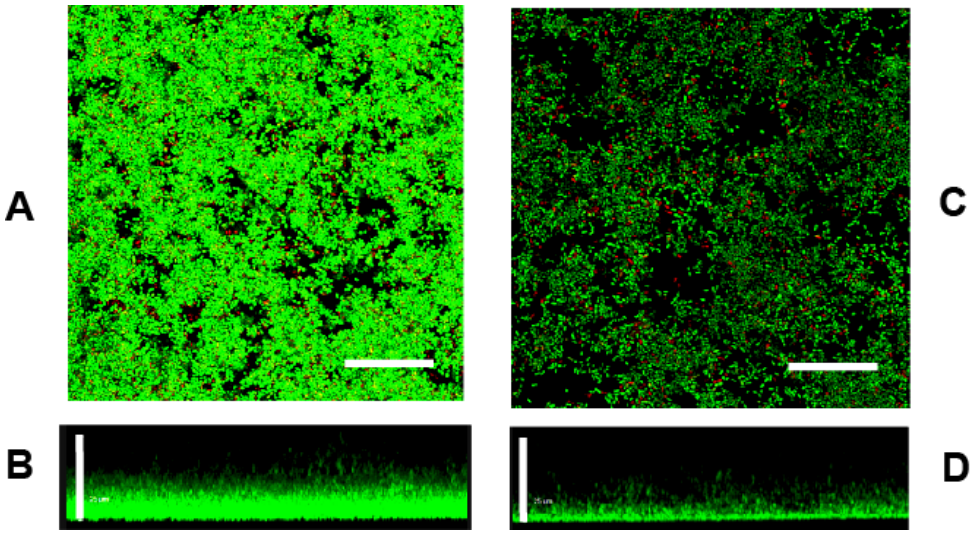

Figure 3: Bacterial biofilms observed by confocal microscopy. Bacteria are allowed to adhere to a solid plastic surface where they grow and connect among themselves. After development of the biofilm, the bacteria are stained with dyes that allow differentiating between live cells (green fluorescence) and dead ones (red fluorescence). A young actively growing biofilm with a small amount of dead cells is shown in the left panels, whereas an old biofilm disintegrating is depicted to the right. Note the density (panels **A** and **C**), and the thickness (panels **B** and **D**) of both biofilms. The photographs show three-dimensional reconstructions of horizontal (**A**, **C**) and vertical (**B**, **D**) scans (40 at the x–y plane, and 68 scans at the x–z plane). The scale bar is 25 µm.

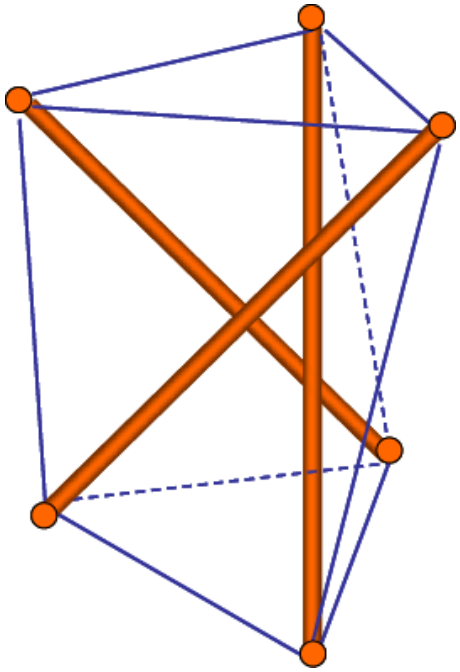

Figure 4: A minimal tensegrity system is generated by three struts (orange) that are interconnected by cords (blue). There is a continuum of communication between the participating elements and the system is held together by tensegritic forces. A single cut in one of the communicating strings would result in the collapse of the system that, otherwise, is able to support a substantial weight (author's drawing modified from http://www.tensegriteit.nl/e-simple.html).

concept of ***biomolecular tensegrity*** that will define the regulatory features of bacterial networks. Under these assumptions, we may consider bacterial cells as built like units (bits) that respond to external stimuli, and that they respond to stimuli by sending intra- and inter-cellular signals that trigger concerted responses, that is to say an interconnection (tensegritic system) between members. In this sense, the usual concept of whether bacteria are or not pathogenic would be irrelevant, because in many cases, pathogenicity is due to either an opportunistic behaviour or to the niche they colonize. In this sense, it is relevant to point out that commensal bacteria could express virulence-related proteins when the populations are growing in biofilms [76]. In other cases, the bacteria would take advantage of either immuno-compromised humans or the environment in which they are located, like the nosocomial infections. These instances could be taken as responses of tensegrity systems to maintain their integrity.

Conclusions

The understanding of the mechanistic processes involved in global gene expression either at a single cell or at community levels, and their regulation will help to develop successful approaches to combat AMR, but

we have arrived in the post-"omics" era in a somewhat embarrassing position. We know the genomic content of a large number of bacteria and the gene expression patterns of many bacteria under different environmental conditions. However, only for a minority of gene products whose cellular function is presently known we do understand how they work and/or how they interact with other components in the cell. This situation is equivalent to being able to understand a code, but not the language of the person sending us the message. To design strategies to combat AMR, we have to try to decipher the message by exploring ways to understand the language. For the past two decades we have generated a huge amount of data that has vastly exceeded our capacity to interpret and to make use of the resulting information. The need to separate valuable from superfluous data is becoming urgent if we want to get reliable source of information. The employment of the so-called "big data approaches" in conjunction to data mining and search engines is mandatory. Fortunately, research in bacteria allows highly sophisticated genetic analyses, complex and sensitive physiological measurements, and detailed biochemical and biophysical studies of the components of regulatory systems. It is within the bacterial world that we are starting to understand the relationships between networks of communication and global regulatory systems. Thus, the goal of achieving a basic understanding of all the biological molecules within a cell and their interactions is now faintly visible on the horizon.

Considering the seriousness of the problem that is ahead of us, there is a need for a consensual approach on new strategies to control bacterial virulence and the transfer of genetic traits related to AMR. In this regard, it would be most interesting to: i) explore the virulence-activating genes regulators as targets for the selected diseases, ii) evaluate the possibility of controlling these diseases by using novel approaches, iii) communicate to the general public about the threat posed by these diseases, iv) promote awareness in target hospitals and research centres on the importance of tackling these diseases, and v) to provide an open space for discussion of approaches and findings.

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