EDITORIAL

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Acquisition and horizontal diffusion of β-lactam resistance among clinically relevant microorganisms

Since their introduction into clinical medicine more than 60 years ago, antibiotics have become the main means of controlling bacterial infection. However, the emergence of microorganisms that are resistant to antimicrobial agents has increased and continues to increase at an alarming rate, making the effective control of infectious diseases a major challenge to public health. The emergence of resistance to antibiotics is no more than an unavoidable and perhaps irreversible consequence of bacterial adaptation to the selective pressure of antibiotics. The unlimited and indiscriminate use of antibiotics over long periods of time in biological systems, such as humans, other animals, and/or plants, has allowed and even promoted the emergence and spread of resistance to antibiotics. Antimicrobial agents have been found in sewage, and they later contaminate rivers, lakes, and oceans. This interconnection between ecosystems promotes a strong selective pressure, altering the environment and advancing the emergence and spread of resistant microorganisms.

β-Lactams are the most varied and widely used of all the groups of antimicrobial agents in human and veterinary medicine. They act by inhibiting the final phase of bacterial cell-wall synthesis. These drugs have little toxicity and a broad therapeutic margin. Penicillins and cephalosporins, which are based on 6-aminopenicillanic acid and 7-aminocephalosporanic acid, respectively, are the two classical β-lactam families. Moreover, β-lactams such as monobactams and carbapenems have also been developed. Although β-lactams are the treatment of choice for a large number of infections, the progressive emergence of acquired resistance has limited their use and their efficacy in certain situations.

Bacteria may become resistant through several mechanisms: production of enzymes that inactivate an antibiotic, alteration of the target (the bacterial molecule with which the antimicrobial agent interacts), alteration of permeability, and activation of trans-membrane efflux (active pumping out). Mutations of cellular genes, acquisition of exogenous resistance genes, or a combination of these two events are responsible for these mechanisms. A good example of acquired resistance due to point mutations is provided by the acquired resistance to nearly all cephalosporins and monobactams in some gram-negative bacteria due to the derepression of a chromosomal cephalosporinase encoded by the β-lactam-inducible *ampC* gene. Organisms such as *Enterobacter* spp., *Citrobacter freundii*, *Serratia marcescens*, *Morganella morganii*, and *Pseudomonas aeruginosa* express low levels of an AmpC β-lactamase; however, the enzyme is induced in response to β-lactams (e.g., cefoxitin or imipenem). AmpC β-lactamases are active-site serine enzymes that are primarily

cephalosporinases. Induction of *ampC* expression appears to involve several gene products associated with this pathway, including AmpR, AmpD, and AmpG. Organisms expressing these enzymes are not resistant to third-generation cephalosporins unless the AmpC β-lactamase is expressed at high levels. Point mutations in one or some of these regulatory genes alter the expression of AmpC β-lactamase and may promote the above-mentioned resistance pattern. Other examples are the acquisition of resistance to carbapenems due to point mutations that alter the permeability by altering the expression of certain porins, such as OprD of *P. aeruginosa*, or to the presence of mutant bacteria that over-express efflux pump genes. Efflux systems that contribute to antibiotic resistance have been described in a number of clinically important bacteria, including *P. aeruginosa*, *Campylobacter jejuni*, *Escherichia coli*, *Streptococcus pneumoniae*, *Salmonella enterica*, and *Staphylococcus aureus*.

There are many examples in the literature of gene acquisition. One of the most evident examples is the acquisition of degrading enzymes, such as aminoglycoside-modifying enzymes or β-lactamases. Another example is the acquisition of new targets that are resistant to antimicrobial action, such as the *mecA* gene by *S. aureus*. This acquired gene confers resistance to all β-lactam antibiotics by the production of a new penicillin-binding protein, PBP2′ (PBP: transpeptidase, transglycosylase, and carboxypeptidase enzymes that manufacture the peptidoglycan of the bacteria). *S. aureus* carrying PBP2′ are named methicillin-resistant *S. aureus* (MRSA), and they are clinically relevant microorganisms due to their pathogenic end epidemic features.

Finally, acquisition of a resistance gene and its evolution by mutation is frequently observed among *Enterobacteriaceae* and β-lactamases. The wild-type *E. coli* strain is susceptible to aminopenicillins, cephalosporins, and the association amoxicillin–clavulanic acid (an inhibitor of β-lactamases). However, about 60% of *E. coli* strains are nowadays resistant to aminopenicillins due to production of plasmid-mediated β-lactamases, such as TEM-1, TEM-2, and SHV-1, known as broad-spectrum β-lactamases. Although these strains remain susceptible to cephalosporins and amoxicillin–clavulanic acid, the acquired genes can mutate and a single nucleotide alteration (mainly in amino acid 238) can spread resistance to aminopenicillins and cephalosporins. These newly derived enzymes are called extended-spectrum β-lactamases (ESBL) due to their increased spectrum of activity. Mutations of the broad-spectrum TEM-type enzymes (mainly in amino acid 69) can also confer resistance to β-lactamase inhibitors, such as clavulanic acid, but not to cephalosporins. These enzymes are named inhibitor resistant β-lactamases.

The above-mentioned genes can spread rapidly within a bacterial population and from one environment to another by sharing and exchanging genetic information. Resistance between bacteria may be transferred by several mechanisms. When the selective pressure of antibiotics is exerted, bacteria already have a large population of resistance genes available to them. This provides an environment where the development and spread of antibiotic resistance is likely to continue indefinitely, both due to the selective pressure of antibiotics and to resistance already present in the population.

The principal mechanisms by which transfer and uptake of resistance can occur are transformation, transduction, and conjugation. Bacteria can pick up naked DNA from their environment through the process of transformation. The DNA may come from a variety of sources, but the most frequent is remnants from dead bacterial cells. In transformation, a cell-surface receptor binds to DNA, which is then transported across the membrane and one strand of the DNA is digested away. The DNA that enters the cell is thus single-stranded. As long as the incorporated DNA is sufficiently homologous to the host DNA, recombination occurs and the new DNA will replace a strand of the host DNA. If the new DNA is of a different allelic nature than the host DNA, a gene conversion event can occur.

β-Lactams resistance in *S. pneumoniae* (gene *pbp2X*, *pbp2B*, and *pbp1A*), *Helicobacter pylori* (gene *pbp1A*) or neisseriaceae (gene *penA*) due to subsequent recombinations of different DNA fragments are examples of acquisition of resistance by transformation and recombination. These DNA fragments confer a gene mosaic pattern to their PBPs, resulting in a low affinity for β-lactam antibiotics. Similarly, there are some examples for other non-β-lactam drugs, such as quinolones in *S. pneumoniae* (mosaic patterns of *gyr*A and *par*C genes), tetracycline in *Gardnerella vaginalis*, *Mycoplasma hominis*, or *Ureaplasma urealyticum* (gene *tet*M), and the resistance to sulfamides and trimethoprim in general (*sul* and *dfr*).

Bacteriophages provide one of the most efficient vehicles for moving DNA sequences between bacterial cells. One consequence of transduction is the dissemination of sequences allowing bacteria to become more pathogenic and antibiotic resistant. Diffusion of resistance by transduction was first documented by the diffusion of the enzyme penicillinase within *S. aureus*. Although the gene that codifies this penicillinase is present within a plasmid, the plasmid is small enough to be packaged entirely within a viral particle, which explains its rapid diffusion among *S. aureus* strains. This resistance is now present in about 95% of all clinically relevant *S. aureus* strains, as well as in other *Staphylococcus* species. Moreover, the occurrence of phage particles carrying sequences of bla_{OXA}- and bla_{PSE}-related genes that encode β-lactamases has recent-

ly been demonstrated in sewage. The potential contribution of phages to the spread of some β-lactamase genes therefore seems quite likely.

Conjugation, first discovered in 1946 by Lederberg and Tatum, is another method that is very adaptable and efficient for intra- or inter-species genetic transfer. This process involves direct cell-to-cell contact of two bacterial cells and the subsequent transfer of DNA. Conjugation can occur between species that are unrelated; for this reason, a large gene pool is available with which bacteria can exchange and acquire new genetic material. Sex pili make contact between the donor and the recipient cell in gram-negative bacteria. Once the two cell walls are in contact, a mating bridge is formed. The plasmid DNA in the donor, which may contain antibiotic resistance genes, is nicked in one strand; this strand proceeds into the recipient cell by undergoing rolling-circle replication. Complementary copies of the DNA are produced in both the donor and the recipient cells. Finally, the linear plasmid in the recipient becomes circular and both cells then have a copy of the plasmid.

Since their discovery in the late 1950s, antimicrobial resistance in both gram-negative and gram-positive bacteria have been increasingly associated with plasmids. It has been documented that many of the β-lactamase genes detected within plasmids have a chromosomal origin. Some examples are the chromosomal AmpC β-lactamases present in the above-mentioned species that are now found in plasmids. Similarly, some ESBL originate from chromosomal enzymes, such as SHV-1 of *Klebsiella pneumoniae* or the CTX-M enzymes derived from different species of *Kluyvera*. Nowadays, plasmid-mediated diffusion of β-lactamases is of great concern and contributes to the enormous diffusion of this kind of enzyme throughout the microbial world. About 60% of *E. coli* strains are now considered to carry a TEM-1, TEM-2, or SHV-1 enzyme. Broad-spectrum β-lactamase derivatives and other ESBL that appeared in the mid-1980s are increasing among *E. coli* strains (and other species). These enzymes drastically reduce the therapeutic options to treat infections. The reservoir of such enzymes might be the commensal microbiota of healthy individuals and not only patients undergoing antibiotic treatment.

Gene-transfer mechanisms provide a starting point for other, more complex mechanisms of development and spread of resistance, such as mobile genetic elements (other than the previously mentioned bacteriophages and plasmids). These elements allow mobilization of specific DNA fragments from one region to another, from plasmids to plasmids, from chromosome to chromosome, and between plasmids and chromosomes. The most relevant transposable elements are insertion sequences, transposons, and gene cassettes of integrons.

Insertion sequences and transposons are similar structures that differ in size and gene content. Insertion sequences are small elements that encode only their capacity for replicative recombination, whereas transposons are larger, apparently composite elements terminated by insertion sequences and containing genes within the central region (frequently antimicrobial resistance genes). Transposable elements cannot exist as free particles in a bacterium. They are integrated in the bacterial genome or in the genetic material of a plasmid or a prophage. They have the ability to move between these sites using an enzyme called transposase.

The participation of transposable elements in the diffusion of β-lactamases has been widely observed, with the CTX-M family of ESBL being the most frequently studied. As mentioned above, most CTX-M enzymes seem to originate from the chromosomal genes of different *Kluyvera* species and have been described within conjugative plasmids. Moreover, many of these genes have been associated with insertion sequences or transposons, such as IS*10*, IS*26*, IS*Ecp1*, and IS*903*-like and Tn*21*-related transposons.

The other elements that have been also associated with β-lactamases are integrons. Integrons are defined as elements containing the genetic determinants of the components of a site-specific recombination system that recognizes and captures mobile gene cassettes. These gene cassettes could be resistance genes and frequently are genes that codify for β-lactamases. An integron includes both a gene encoding an integrase and an integration site for the gene cassette. Finally, following the earlier identification of bacterial conjugation, research has revealed numerous combinations of genetic modules derived from bacteriophages, plasmids, and transposons. These modules generate conjugative and mobilizable transposons as well as genomic islands.

In conclusion, gene transfer is a widespread phenomenon that may occur in a variety of ways. A good example is the acquisition and diffusion of β-lactam resistance, an event that drastically limits treatment options. Even if selective pressure by antibiotics were reduced, resistance could still spread through the population in ways such as those described above. A wide, multidisciplinary approach is therefore needed to control the spread of resistance genes not only in bacteria, but also among fungi, viruses, and parasites.