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UNDERSTANDING THE DISEASE

Understanding resistance

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Introduction

The ability of bacterial pathogens to develop resistance against antimicrobial drugs is a key issue when caring for infected patients, especially those admitted to the intensive care unit (ICU). Antimicrobial resistance increases the risk of inadequate empirical coverage and may dramatically complicate the management of definite therapy, which often translates into worsened outcome for the most severely ill patients (Fig. 1) [1]. Nowadays, resistance rates have reached alarming levels, notably in ICU-acquired Gram-negative bacilli (GNB), and current trends suggest that the magnitude of the problem will continue to rise [2]. In this context, understanding resistance mechanisms may serve as a prerequisite to optimize antimicrobial use in daily practice and to appraise how reducing antibiotic consumption could help control the spread of multidrug-resistant (MDR) pathogens.

Intrinsic resistance mechanisms

Virtually all bacterial species exhibit intrinsic resistance to certain antimicrobial classes. This wild-type phenotype depends on chromosomal genes from the core genome of the species and is transmitted vertically during cell duplication [3]. A variety of innate mechanisms may prevent a drug from reaching its action site, including enzymatic inactivation (e.g., hydrolysis of beta-lactams in the periplasmic space by chromosomal penicillinases or inducible AmpC cephalosporinases in some *Enterobacteriaceae* species), impermeability (e.g., inability of glycopeptides to penetrate the outer membrane of GNB, or lack of oxidative metabolism to drive cellular uptake of aminoglycosides in anaerobic bacteria), and extrusion from the bacterial cell through efflux systems expressed at low level. Then, natural resistance may rest on an intrinsically weak affinity between the antibiotic and its usual target, or the lack of target. This is particularly relevant for beta-lactams whose spectrum of activity stems from their capability to inhibit the natural penicillin-binding proteins (PBPs) of each given species; for instance, aztreonam displays no affinity for the PBPs of Gram-positive bacteria. This equally applies for polymyxins, a class that exert its bactericidal effect through disrupting the outer membrane by binding the lipid A moiety of lipopolysaccharide (LPS): these drugs are inactive against Gram-positive rods—which lack an outer membrane—and several GNB species producing LPS with poor polymyxin-binding properties such as *Proteus* spp., *Serratia* spp., *Morganella* spp., or *Burkholderia* spp. [4]. As a whole, intrinsic resistances can be easily circumvented using antimicrobial agents from the routine armamentarium and are not challenging for the treatment of severe infections. Acquired mechanisms account for the widest part of the burden.

Acquired mechanisms: how does resistance emerge?

Acquired resistances may first result from chromosomal mutations which either promote the transcription of resistance genes naturally expressed at low level or modify the antibiotic target, thus increasing minimal inhibitory concentrations (MIC) up to values that preclude the therapeutic use of the drug. These mutations are not directly prompted by antimicrobial exposure but occur randomly during the DNA replication process inherent to cell synthesis. In GNB with inducible AmpC cephalosporinase such as *Enterobacter* spp. or *Pseudomonas aeruginosa*, mutations in the regulatory genes of bla_{ampC} can lead to its permanent overexpression, thereby unbalancing the enzyme/substrate ratio and conferring resistance to penicillins and extended-spectrum cephalosporins [2]. Mutations in DNA gyrase- and topoisomerase IV-encoding genes (*gyrA* and *parC*) gradually lessen the capacity of fluoroquinolones to bind and inhibit these enzymes. Mutation-based modifications of LPS may

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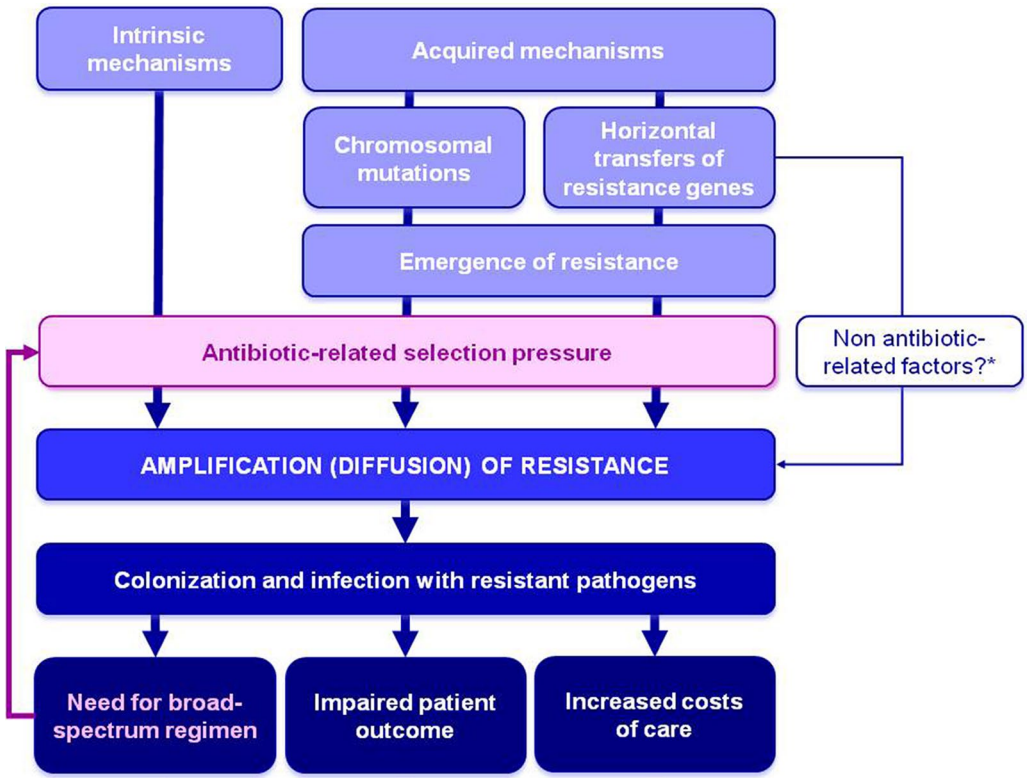

Fig. 1 Contributing factors and consequences of antimicrobial resistance: a schematic view. *Selected examples: (i) resistance-encoding plasmids may also encode toxin–antitoxin systems that force their maintenance in the recipient bacteria ("resistance addiction"), independently of the selective advantage conferred in case of antibiotic exposure—this factor could contribute to the persistence and dissemination of CTX-M enzymes in community populations with a relatively low antibiotic selection pressure [14]; and (ii) in methicillin-resistant coagulase-negative staphylococci (especially *Staphylococcus epidermidis*) and certain clones of methicillin-resistant *Staphylococcus aureus* (including USA300, a successful lineage of community-acquired MRSA), the mobile genetic element (MGE) carrying *mecA* (namely, Staphylococcal Cassette Chromosome *mec*—SCC*mec*) may be associated with another MGE (Arginine Catabolic Mobile Element—ACME) which enhances bacterial fitness and survival on skin and mucosal surfaces [15], thereby favoring their implantation in these ecosystems

reduce polymyxin susceptibility in *Klebsiella pneumoniae* and *Acinetobacter baumannii* [4], while vancomycin MIC may increase in staphylococci following various alterations of the cell-wall metabolism (including amplification of D-Ala-D-Ala residues or reduced peptidoglycan turnover). Next, chromosomal mutations can induce structural changes in outer membrane porins that impede the drug from entering the cell, such as in *P. aeruginosa* mutants with selective imipenem impermeability due to the functional loss of oprD [5]. Moreover, mutations in the regulatory genes of active efflux pumps may heighten their expression to a level that maintains drug availability at the action site below the effective threshold. This mechanism stands as a major driver of multidrug resistance in *P. aeruginosa* and *A. baumannii* [2]. An essential feature is that different antimicrobial classes may be substrates of the same pump; therefore, even a single-drug exposure (e.g., a beta-lactam) may select mutants with an MDR phenotype (e.g., resistance to beta-lactams plus fluoroquinolones and/or aminoglycosides). Overall, multidrug resistance can emerge in non-fermenting GNB on the sole basis of sequential chromosomal mutations—with no obvious negative impact in terms of fitness and virulence [6]—whereas it usually implies the acquisition of exogenous genetic material in *Enterobacteriaceae*.

Resistance genes may be horizontally transferred from a donor to a recipient bacterial cell according to three distinct routes [3]: (1) transduction, i.e., gene exchange via bacteriophages (viruses that infect bacteria on a species-specific mode)—this mechanism could contribute to the acquisition of methicillin-resistance in *Staphylococcus aureus* through the chromosomal integration of *mecA* [7], a gene which encodes an additional PBP (i.e., PBP2a) with minimal affinity for all beta-lactams except fifth-generation cephalosporins; (2) transformation, whereby free foreign DNA is assimilated and incorporated into the chromosome—in *Streptococcus pneumoniae*, PBP-encoding genes recombine with those of other species

from the *Streptococcus mitis* group, yielding mosaic PBPs with reduced affinity for penicillins; and (3) conjugation of plasmids, which are circular, double-stranded extrachromosomal DNA structures comprising an autonomous replication system. Plasmids are responsible for the acquisition of penicillinase-encoding genes in several important pathogens, including *S. aureus*, *Haemophilus influenzae*, and *Escherichia coli*, and play a central role in the on-going pandemic of extended-spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* [1, 2]. ESBL derive from either narrow-spectrum penicillinases of unknown origin (e.g., TEM) or chromosomal beta-lactamases intrinsically found in *Enterobacteriaceae* (e.g., SHV from *K. pneumoniae*, or CTX-M from the environmental *Kluyvera* spp.). All types hydrolyze third- and fourth-generation cephalosporins, while most of them remain susceptible in vitro to inhibitors such as tazobactam. The epidemic properties of certain plasmids foster intra- and interspecies transfers of ESBL-encoding genes, and are massively involved in the rapid spread of CTX-M in both community-acquired and healthcare-associated strains of *E. coli*. The global dissemination of plasmid-borne carbapenemases now represents an additional level of threat, in *Enterobacteriaceae* as in non-fermenting GNB [2]. These enzymes are part of various classes (serine beta-lactamases and metallo-beta-lactamases, e.g., KPC and NDM-1 carbapenemases, respectively) but share the ability to hydrolyze most of the currently available beta-lactams, including carbapenems. Plasmids can equally encode resistance determinants to non-beta-lactam drugs, particularly aminoglycosides (aminoglycoside-modifying enzymes and rRNA 16S methylases), fluoroquinolones (Qnr proteins, efflux pumps, and the inactivating enzyme AAC(6′)-Ib-cr), and even polymyxins (alterations of lipid A by MCR-1, a phosphoethanolamine transferase) [8]. Strikingly, plasmids may aggregate resistance genes for several antimicrobial classes; in this case, a single conjugation event suffices to provide an MDR phenotype to the recipient strain. Distinct resistance-determining plasmids may also be acquired by the same bacterium. Major concerns have especially been raised following recent reports of *Enterobacteriaceae* strains co-carrying plasmid-borne *mcr-1* and carbapenemase-encoding genes [9].

Antibiotic consumption as a promoter of resistance

Antimicrobial exposure selects rather than creates resistant bacteria. Indeed, genetic events underlying the emergence of resistance are mostly spontaneous, and selection pressure will only enable a resistant isolate to survive and amplify within a more susceptible bacterial population. In this respect, antibiotic-related damage in the anaerobic gut flora and other commensal microbiomes compromise colonization resistance to MDR pathogens, thereby easing their implantation and a rise in colonization densities, which might in return predispose to subsequent invasive infections and cross-transmission [10]. This impact appears cumulative, notably for carbapenems or metronidazole, since each supplementary daily dose correlates with the likelihood of intestinal carriage of MDR GNB in critically ill patients [5, 11]. Hence, preserving colonization resistance at the patient level through de-escalation (whenever possible) and other antibiotic stewardship initiatives is probably of pivotal importance when tackling the global menace of multidrug resistance. Interestingly, a recent publication indicates that restoring a "normal" intestinal flora through fecal transplantation is effective in the eradication of resistant pathogenic organisms and elimination of resistance genes in patients with recurrent *Clostridium difficile* infection [12]. Whether selective gut decontamination with non-absorbable antibiotics may help clear MDR pathogens warrants further investigations [2]. Last, assuming that the hospital setting evolves as an open ecosystem owing to patient movements, reducing antibiotics consumption in community-based populations should be viewed as a compulsory axis of intervention to limit the influx of transmissible resistance genes in this environment. This applies for both primary care and antimicrobial misuse in the food industry [13]—the hottest example being undoubtedly the link between polymyxins use for agricultural purposes and the emergence and spread of plasmid-borne colistin resistance in *Enterobacteriaceae* [8].

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Compliance with ethical standards

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