MULTIDRUG-RESISTANT AND EXTREMELY DRUG-RESISTANT BACTERIA: ARE WE FACING THE END OF THE ANTIBIOTIC ERA?

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Abstract. Antibiotics are one of the most significant advancements of modern medicine. They have changed the prognosis of several bacterial infections, and made possible advanced medical practices associated with a high risk of infectious complications. Unfortunately, antibiotics are affected by the phenomenon of antibiotic resistance, which jeopardizes their efficacy. In recent years, antibiotic discovery and development has been lagging, due to a lower appeal of this sector for the pharmaceutical industry, while antibiotic resistance has continued to evolve with the eventual emergence and dissemination of bacterial strains which are resistant to most available antibiotics and pose a major challenge to antimicrobial chemotherapy. This worrisome scenario, indicated as the "antibiotic resistance crisis", has been acknowledged by Scientific Societies and Public Health Agencies, and is now gathering an increasing attention from the Media and Governments. This article reviews the antibiotic-resistant pathogens which currently pose major problems in terms of clinical and epidemiological impact, and briefly discuss future perspective in this field.

Key words: Antibiotics; antibiotic resistance; multidrug resistance.

INTRODUCTION

Antibiotics have represented one of the most significant discoveries of the twentieth century. They have dramatically changed the prognosis of bacterial infections (*e.g.* pneumonia, sepsis, meningitis, wound infections, cellulitis, typhoid fever, syphilis) by reducing morbidity and mortality due to these infections. Antibiotics have also made possible a number of medical practices that would otherwise be precluded by an exceedingly high risk of infectious complications, such as "contaminated" surgery, implantation of prosthetic devices, solid organ and stem cell transplantation, deep immune suppression treatments. Losing antibiotics due to bacterial resistance, therefore, is a very serious issue that can revert the morbidity and mortality by infectious diseases to that typical of the pre-antibiotic era, and jeopardize the success of several advanced medical practices. This has recently been acknowledged by the WHO, in a report on the global impact of antimicrobial resistance [1]. The growing and challenging menace of antibiotic resistance to public health has also been underscored in a recent report, commissioned by the government of the United Kingdom, where it has been estimated that by year 2050, antibiotic resistance could cause approximately 10 millions of deaths annually [2].

The phenomenon of antibiotic resistance reflects the ability of bacteria to evolve resistance mechanisms by which the bacterial cell can escape the lethal action of antibiotics. Well known examples of antibiotic resistance mechanisms include: i) production of enzymes that inactivate antibiotics (*e.g.* β-lactamases, which inactivate β-lactam antibiotics); ii) modification of the molecular target of the antibiotic, so that it is no longer inhibited by the drug while retaining its function; iii) reduction of permeability of the cell envelope to antibiotics, with impaired access of drugs to their intracellular targets; and iv) extrusion of the antibiotic from the bacterial cell by efflux pumps. Evolution of resistance mechanisms in the bacterial cell can be due either to chromosomal mutations or to horizontal acquisition of new resistance genes that encode the resistance mechanisms [3].

The evolution of resistance mechanisms among pathogenic bacteria is part of the normal evolution process, and thus is unavoidable. However, the dynamics of this evolution are strongly influenced by several factors among which the most relevant is the use of antibiotics in clinical, veterinary and agricultural practices. An overuse and misuse of these drugs accelerate the evolution process by increasing the selective pressure for strains that have acquire resistance mechanisms.

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THE ANTIBIOTIC RESISTANCE CRISIS

The phenomenon of antibiotic resistance has been evident since the introduction of antibiotics in clinical practice, and has affected every new antibiotic developed for clinical use. However, until a few years ago, the evolution of antibiotics resistance among pathogenic bacteria was steadily counterbalanced by the discovery and development of new antibiotics active against resistant bacteria. Examples of this saga, occurred between bacteria and humans and ongoing since the 1950s, are represented by the development of penicillinase-stable penicillins (*e.g.* oxacillin, cloxacillin) to address penicillin resistance due to penicillinase production in *Staphylococcus aureus*, of expanded-spectrum cephalosporins (ESC) (*e.g.* cefotaxime, ceftriaxone) to address ampicillin resistance due to broad spectrum β-lactamase production in *Escherichia coli* and other Enterobacteriaceae, and of carbapenems (*e.g.* imipenem, meropenem) to address ESC resistance due to production of extended-spectrum β-lactamases (ESBLs) in Enterobacteriaceae [4].

In recent years, antibiotic discovery and development programs have been lagging behind due to a lower appeal of this sector for pharmaceutical industry [5], while antibiotic resistance has continued to evolve in a relentless manner among clinical pathogens with the emergence and dissemination of bacterial strains which have acquired resistance determinants to several antibiotics and exhibit multidrug-resistant (MDR) or extensively drug-resistant (XDR) phenotypes [6]. These strains remain susceptible to only a few antibiotics and pose a major challenge to antimicrobial chemotherapy. Some strains can end up with being resistant to all the available antibiotics (pan drug-resistant strains) [6], causing untreatable infections.

Such a worrisome scenario, in which the clock is turned back to the pre-antibiotic era and antibiotics are lost to modern medicine, has also been indicated as the "antibiotic resistance crisis" [7]. In the following section, the antibiotic-resistant pathogens which pose major problems and of their clinical and epidemiological impact are briefly summarized and discussed.

THE MAJOR PLAYERS IN THE ANTIBIOTIC RESISTANCE CRISIS

Antibiotic resistance involves most bacterial pathogens. Nevertheless, some species are more prone than others to accrue resistance determinants, and eventually more challenging for antimicrobial chemotherapy. These include *Staphylococcus aureus* and *Enterococcus faecium* among Gram-positives, and Enterobacteriaceae, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* among Gram-negatives.

Methicillin-resistant Staphylococcus aureus, *the first challenging MDR pathogen*

S. aureus is a major pathogen, causing a number of community-acquired and healthcare-associated infections (HAI) that can range from minor cutaneous infections (*e.g.* furuncles) to severe life-threatening infections (*e.g.* pneumonia, severe cellulitis, septicemia and surgical site infections).

Methicillin-resistant *S. aureus* (MRSA), which is resistant to all β-lactams due to the acquisition of a new penicillin-binding protein (PBP) that can take over the functions of all other PBPs and is not inhibited by β-lactam drugs, has been the first challenging antibiotic-resistant pathogen and remains one of the most important in terms of epidemiological and clinical impact [8]. Infections caused by MRSA are associated with increased mortality and healthcare-associated costs, as compared to infections caused by methicillin-susceptible *S. aureus* [9], and the prevalence of MRSA has achieved very high rates in several countries worldwide [10]. In Italy, approximately one third of all *S. aureus* bloodstream infections are caused by MRSA strains according to the data of the European EARS-NET surveillance system (Figure 1) [11], and this proportion has remained overall stable during the past decade.

MRSA dissemination can be effectively controlled by the implementation of rigorous infection control practices in combination with antibiotic stewardship programs. In the Netherlands, where infection control for MRSA has always been aggressive, the prevalence of this resistant pathogen has remained very low, unlike in many other European countries (Figure 1). In England, where in the early 2000s the MRSA proportions were very high, a nationwide campaign to control MRSA has proved to be very effective at reducing MRSA [12], showing that similar campaigns can be effective at curbing the dissemination of this resistant pathogen even in settings of high prevalence.
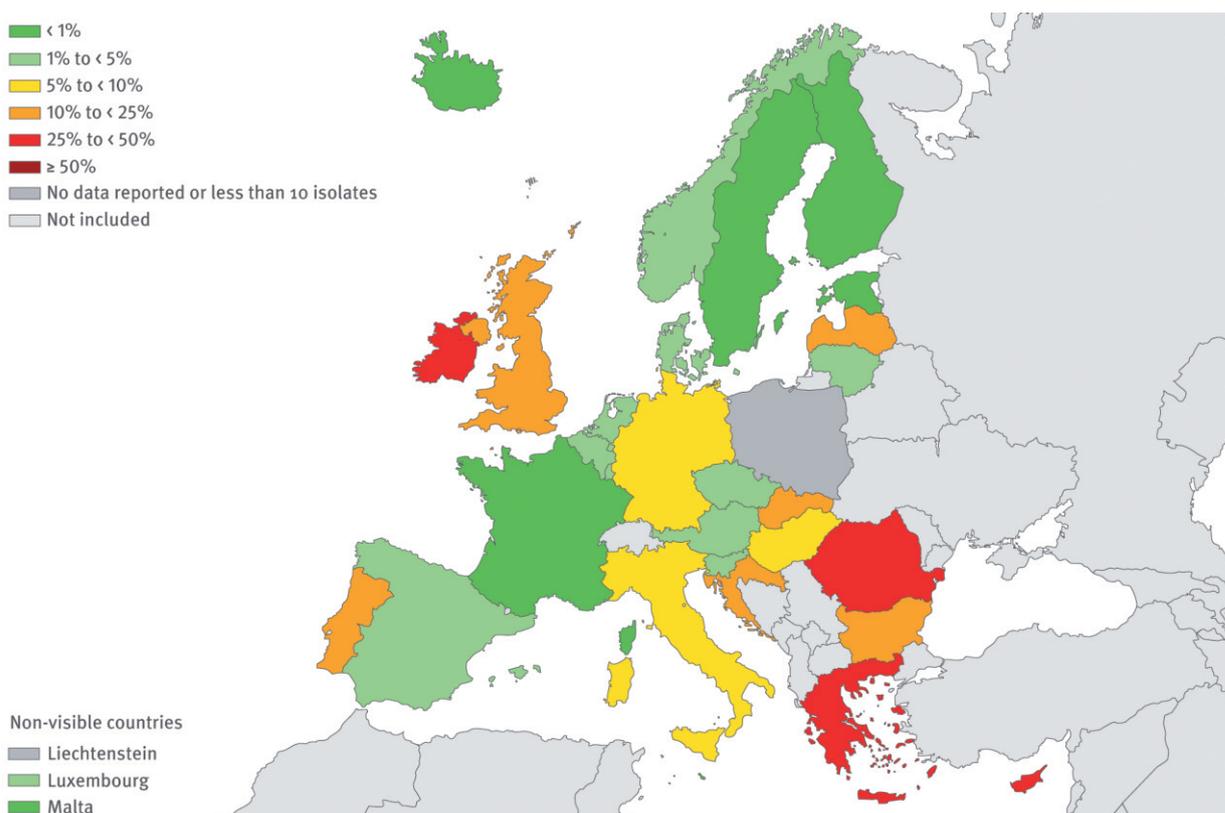
Vancomycin-resistant enterococci: a serious problem in some settings and with some patients

Vancomycin-resistant enterococci (VRE), mostly belonging to the species *E. faecium*, are another group of problem resistant pathogens. VRE strains have developed a complex mechanism to escape vancomycin activity, by modification of their peptidoglycan structure. As such, they remain susceptible to only a few antimicrobial agents (linezolid, tigecycline, quinupristin-dalfopristin) and cause difficult-to-treat infections [13]. Severe VRE infections, including sepsis, are typical of immunocompromised patients, and VRE infections can be an important cause of febrile neutropenia episodes in oncohematology wards [14].

In Europe, the prevalence of VRE is overall lower than that of MRSA, and Italy is not among the most affected countries (Figure 2) [11]. However, a recent increase has been documented by the most recent data from the
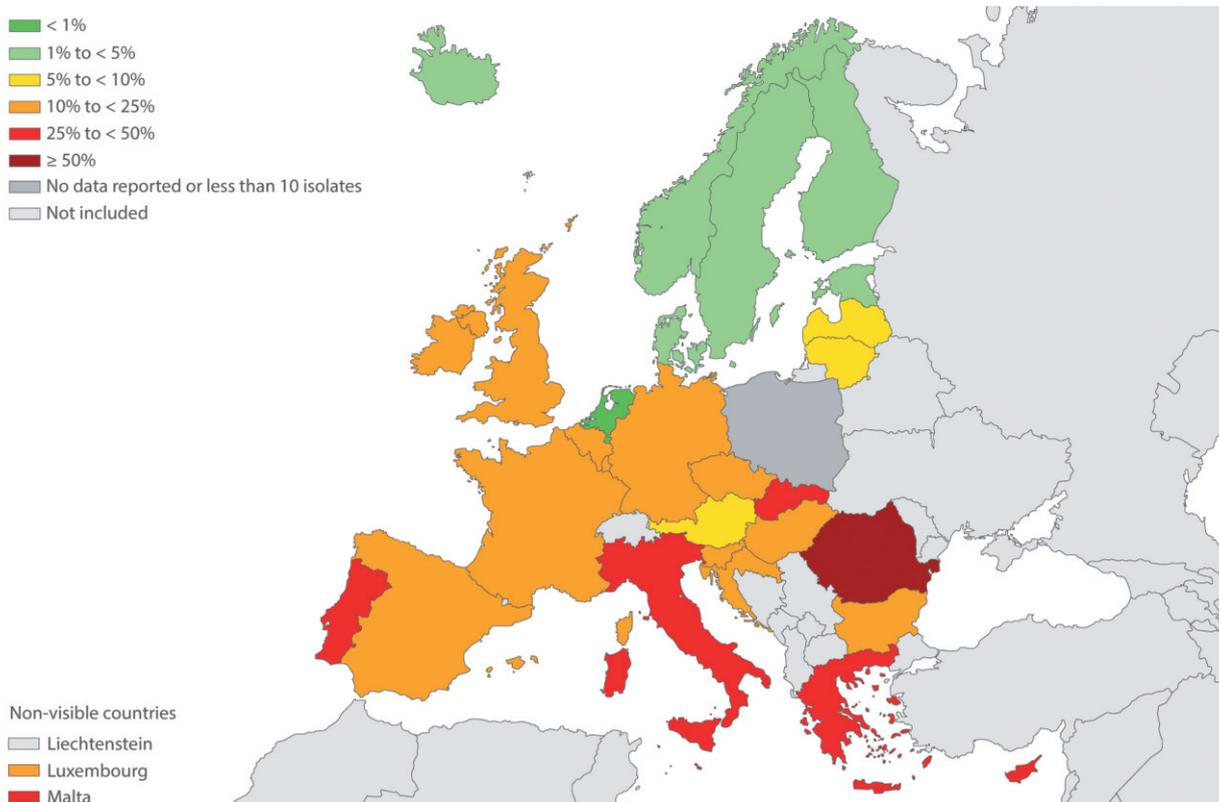
Figure 1. Percentage of methicillin-resistant Staphylococcus aureus among invasive *S. aureus* infections in different European countries from the EARS-NET surveillance system. *Reproduced from: European Centre for Disease Prevention and Control, 2015* [11].

Figure 2. Percentage of vancomycin-resistant *Enterococcus faecium* among invasive E. faecium infections in different European countries from the EARS-NET surveillance system. *Reproduced from: European Centre for Disease Prevention and Control, 2015* [11].

EARS-NET surveillance system [11], suggesting that the importance of VRE should not be discounted.

Pseudomonas and Acinetobacter: the first XDR Gram-negatives causing clinical problems

Pseudomonas aeruginosa is a Gram-negative bacillus living in moist environments, which behaves as an opportunistic pathogen causing infections mostly in hospitalized patients with some impairment of the host defenses. *P. aeruginosa* is a common cause of hospital-acquired pneumonia, including ventilator-associated pneumonia, bacteremia, and catheter-related urinary tract infections occurring in intensive care units (ICU). It is also an important cause of bacteremia in neutropenic patients, and a major cause of burn infections and of chronic respiratory tract infections in cystic fibrosis patients.

P. aeruginosa is naturally resistant to several antibiotics, and exhibits a remarkable propensity to acquire resistance to the various anti-*Pseudomonas* agents. Among Gram-negatives it has been the first species to present serious problems of multi drug resistance [15]. Of particular concerns are strains exhibiting an XDR phenotype including all anti-*Pseudomonas* agents except colistin (the so-called colistin-only susceptible strains), which are very difficult to treat and dangerously close to pan-drug resistance [15]. The prevalence of these strains is not negligible (5-10% in recent surveillance studies) [16]. Some of these strains are now treatable with ceftolozane-tazobactam, a new anti-*Pseudomonas* β-lactam antibiotic that has been very recently approved for clinical use [17]. However, this new drug is not active against strains producing metallo-β-lactamases, which are often susceptible only to colistin and remain a serious clinical problem [17].

Acinetobacter baumannii is a Gram-negative coccobacillus living in the environment, which also behaves as an opportunistic pathogen in hospitalized patients with impaired host defenses. *A. baumannii* can be an important cause of ventilator-associated pneumonia in ICU patients, and can also be responsible of healthcare-associated bacteremia and skin and soft tissue infections [18].

Similar to *P. aeruginosa*, *Acinetobacter* is naturally resistant to several antibiotics and prone to acquire resistance to the relatively few agents that can be used for treating *Acinetobacter* infections. The major issue in this case is represented by resistance to carbapenems, since these drugs are the front-line agents for treating severe *Acinetobacter* infections and carbapenem-resistant *A. baumannii* (CRAB) strains have usually acquired resistance also to the other anti-*Acinetobacter* agents except colistin and, in some cases, tigecycline [19].

In CRAB strains, resistance is usually due to the production of carbapenemases, *i.e.* β-lactamases capable of degrading carbapenems. Successful clones of CRAB producing the carbapenemases OXA-23 or OXA-58 have recently disseminated globally and, in some settings, have largely replaced carbapenem-susceptible *Acinetobacter* strains. In Italy and other Mediterranean countries, according to the most recent data from the European EARS-NET surveillance systems, the majority of *Acinetobacter* strains isolates from invasive infections are CRAB [11], which are causing serious problems in several hospitals.

Carbapenem-resistant Enterobacteriaceae: the ultimate challenge of antibiotic resistance

The family Enterobacteriaceae includes several important pathogens (*e.g. Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Enterobacter* spp., *Serratia marcescens*, *Citrobacter* spp., *Salmonella enterica*, *Providencia* spp., etc.) which are overall a common cause of bacterial infections, either in the community and in the hospital setting. A recent point-prevalence survey on healthcare-associated infections (HAIs) in Europe, promoted by the ECDC, revealed that Enterobacteriaceae are the most common cause of HAIs in European hospitals, accounting for more than one third of cases, with *E. coli* and *Klebsiella* being the most important species [20].

Evolution of antibiotic resistance in these species during the past decade has been very rapid and challenging. Originally susceptible to fluoroquinolones and ESC, which were the front-line agents for enterobacterial infections, *E. coli* and *Klebsiella* have rapidly developed resistance to these agents. The global-scale dissemination of ESBLs, in particular, has played a major role in the evolution of resistance to ESC in *E. coli* and *Klebsiella*, with a steady increase of resistance rates that have achieved remarkably high values [11]. These epidemiological circumstances have caused an increased use of carbapenems, given the efficacy of these drugs for the treatment of severe infections caused by ESBL-producing Enterobacteriaceae, followed by a "falling dominoes" effect leading to the emergence and dissemination of carbapenem-resistant Enterobacteriaceae (CRE). CRE are mostly contributed by carbapenem-resistant strains of *Klebsiella pneumoniae* which have acquired carbapenemases of the KPC, VIM, NDM, or OXA-48 type. These strains have a remarkable ability to disseminate in healthcare settings, and have rapidly attained a high-level endemicity in several areas, including Italy [11,21]. This high propensity for dissemination, along with the invariably MDR and often XDR phenotype of CRE strains and the high morbidity and mortality associated with CRE infections, account for the remarkable clinical and epidemiological impact of these strains [22]. Colistin remains one of the few antibiotics active against CRE, and this old drug that had virtually been abandoned since the 1970s due to toxicity issues has now been "rediscovered" as a last-resort agent for treatment of infections caused by XDR strains of CRE and other Gram-negatives [23]. The increased use of colistin has rapidly been followed in turn by a "falling dominoes" effect leading to the emergence and dissemination of colistin-resistant strains of CRE [24,25], further narrowing

the treatment options that remain available for CRE.

The mechanisms of resistance to colistin in CRE strains has recently been investigated by means of whole genome sequencing technologies, and it has been shown that several chromosomal mutations can be responsible for upregulating the endogenous bacterial systems that modify the lipid A colistin target reducing its affinity for polymyxins [26,27]. More recently, a transferable plasmid-mediated gene encoding an enzyme responsible for colistin resistance by lipid A modification has also been discovered [28], raising a considerable concern.

CONCLUSIONS AND FUTURE PERSPECTIVES

The emergence and dissemination of XDR pathogens which remain susceptible to only a few antibiotics, with the occasional detection of PDR strains, has definitely led modern medicine dangerously close to face the end of the antibiotic era. Awareness of this antibiotic resistance crisis has emerged gradually, and the responses have initially been rather slow and partial. However, the call to action eventually prompted several initiatives to address this problem, including a new thrust to programs for discovery and development of new antibiotics.

The fact that MRSA has emerged first as a resistant pathogen of clinical and epidemiological relevance explains the earlier efforts at finding new drugs active against this pathogen. These efforts, started since the mid 1990s, have led to the relative large repertoire of anti-MRSA antibiotics that recently became available for clinical use against this pathogen (*e.g.* linezolid, tigecycline and daptomycin in the 2000s, and more recently ceftaroline, ceftobiprole, telavancin, dalbavancin, oritavancin and tedizolid) [29].

The efforts at discovery and development of new drugs against resistant Gram-negatives have started later, and the anti-Gram-negative pipeline is still much thinner and less advanced. A few agents active against XDR *P. aeruginosa* (*e.g.* ceftolozane-tazobactam) or against several CRE strains (*e.g.* ceftazidime-avibactam, imipenem-relebactam, plazomicin) have recently been approved for clinical use or are in the late stages of clinical development, and represent a major breakthrough in the struggle against resistant Gram-negatives. However, even these new agents will not cover all types of resistant Gram-negatives, leaving out *P. aeruginosa* and Enterobacteriaceae producing metallo-beta-lactamases, and CRAB [30].

Under these circumstances, it is evident that to address the antibiotic resistance crisis it is not possible to rely solely upon new drugs, but an integrated strategy is essential, including strict infection control practices and antimicrobial stewardship policies.

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