Le Infezioni in Medicina, n. 4, 301-310, 2017

Update on the main MDR pathogens: prevalence and treatment options

Silvano Esposito, Giuseppe De Simone
Department of Infectious Diseases, University of Salerno, Italy

SUMMARY

In recent years the proportion of multi-drug resistance (MDR) among the bacterial pathogens causing infections, particularly those acquired in healthcare settings has worryingly risen worldwide. It poses a serious public health threat as the multiple patterns of resistance limit the effective treatment options for such infections. Although many bacterial species have developed reduced susceptibility to a wide array of antimicrobial molecules, a particular group of pathogens acronymically referred to as "ESKAPE" *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp*.) plays a clinically relevant role in the aetiology of life-threatening nosocomial infections. In this review, we represent the rise of MDR among the ESKAPE pathogens over the decades and report studies from each continent showing the current prevalence and burden of such infections worldwide.

Keywords: Multidrug-resistance, *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Enterobacter* spp.

INTRODUCTION

Hospital-acquired infections (HAIs) can be defined as the infections contracted in healthcare facilities (including hospitals, nursing homes, clinics and other settings) occurring more than 48 hours after admission. HAIs represent a serious public health threat as they are one of the major causes of morbidity and death in hospitalized patients, leading to prolonged hospital stay and increased hospital charges [1, 2]. The burden of these infections has drastically risen worldwide over last decades. In 2002 the Centers for Disease Control and Prevention (CDC) estimated that the number of HAIs diagnosed in U.S. hospitals was approximately 1.7 million, while the estimated deaths associated with HAIs were 99,000 (most of them due to pneumonia and septicaemia) [3]. In 2011, a large-scale survey conducted on 183 U.S. hospitals reported that 4% of all patients contracted one or more HAIs, therefore the estimated burden of HAI was about 722,000 cases [4]. In 2011-2012 a large study including a total of 273,753 patients from 1149 hospitals through Europe revealed that 5,7% of patients had at least one HAI; this data suggests that the total annual number of HAIs among patients referring to European hospitals was about 3.2 million [5]. Although few studies provide epidemiological data on HAI in the developing countries, it has been estimated that the prevalence of these infections is about 15 cases per 100 patients, thus much higher than that reported from Europe and USA [6]. The management of HAIs is complicated by the growing number of antimicrobial-resistant pathogens to which inpatients are exposed during their staying in healthcare facilities. Indeed, as largely reported in recent literature, different bacterial species become resistant to many classes of antimicrobial and chemotherapeutic agents, thus they have been defined as multidrug-resistant (MDR) [7-9]. Scarce adherence to infection control protocols, inappropriate and excessive use of antimicrobial molecules have contributed to the increase of the MDR phenomenon, that is due to various

Corresponding author
Silvano Esposito
E-mail: silvanoesposito@libero.it

adaptation mechanisms, including biofilm formation, drug inactivation and modification of drug targets [7]; moreover, the spread of antibiotic resistance can be enhanced by mechanisms of horizontal gene transfer among species [8]. In recent years, among the MDR bacterial species many authors have highlighted the clinical relevance of a group of antibiotic resistant pathogens (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter* spp.) acronymically named "ESKAPE". These bacteria represent common causes of life-threatening HAIs, in particular for critically ill patients admitted in intensive care units (ICUs) [7].

Studies of surveillance are of paramount importance as they yield the actual proportion of the MDR problem, moreover, epidemiological studies by providing local data facilitate the efforts to define appropriate control measures in order to check the rise of resistances [10-12].

Enterococcus faecium

The genus *Enterococcus* includes more than 20 species of Gram-positive, facultative anaerobes, bacteria. Amongst the *Enterococcus* species, the most clinically relevant pathogens are represented by *Enterococcus faecium* and *Enterococcus faecalis* [7]. These bacteria can survive for long periods in the hospital setting resisting to the action of some alcohol preparations and thus easily contaminate also the surfaces of medical equipment [13]. Indeed, in the U.S. Enterococci are the fourth most common causative organisms of nosocomial infections, the second cause of bacteraemia and the third cause of urinary tract infections [13-15]. Their ability to develop resistance to many antimicrobial molecules is due to the plasticity of their genomes: they rapidly accumulate genetic mutations and acquire plasmid genes conferring additional resistances. Although penicillin alone or in association with an aminoglycoside has been considered the gold standard against enterococcal infections for decades, most enterococci strains have developed tolerance to the activity of β-lactam antibiotics [13]. Ampicillin resistance has been reported in ~90 % of hospital-isolated *E. faecium* strains and is due to the synthesis of penicillin binding proteins characterized by a low affinity for the penicillins (such as PBP5) [13]. An epidemiological study conducted in several microbiological laboratories throughout The Netherlands found that the rates of ampicillin-resistance among the *Enterococcus faecium* isolated from bloodstream specimens increased from 4% in 1999 to 20% in 2005 [16]. Because of the global emergence of this phenomenon, glycopeptides (*e.g.* vancomycin) have been used for years as reliable alternative to ampicillin for patients with allergy to β-lactams, or in case of infections caused by ampicillin-resistant enterococci [13]. But, once again, several mechanisms of resistance even to vancomycin (such as the acquisition of van gene clusters) were finally reported in Enterococci strains, at first in U.S. hospital settings in late 1980s, then, during the following years, the prevalence of vancomycin-resistant Enterococci (VRE) has worryingly increased worldwide [7, 17]. The number of hospitalizations due to VRE infections in United States increased from 4.60 to 9.48 hospitalizations per 100,000 population during the period 2003-2006 [18]. Currently, CDC estimates that 30% of *Enterococcus* HAIs are attributable to VRE, causing approximately 1,300 deaths each year in the United States [19]. A multicentre prospective study found that in South America the prevalence of vancomycin resistance among all the enterococci isolated was relatively low (6%) compared to that in the United States. In part, it may be due to the fact that in this study *E. faecalis* resulted to be the most frequent species isolated (with a high ratio of *E. faecalis* to *E. faecium*, 5:1) and it is known that the major reservoir of acquired vancomycin resistance is *Enterococcus faecium* whilst *E. faecalis* is characterized by lower antibiotic-resistance [20]. Also in Asia, the proportions of VRE phenomenon are still low: in Thailand, the incidence of VRE never exceeded 5.1% during the decade 1999-2009 [21]. As regards Europe, data from the ECDC Surveillance Atlas revealed that in 2015 VRE rates significantly vary across the continent with some countries reporting <1% of resistant isolates (France, Iceland and Scandinavian countries) whilst the majority of EU countries (including Germany, Italy and UK) have percentages included in the range 10-25%. Only few countries (Croatia, Cyprus, Ireland and Romania) have to front percentages higher than 25% [22]. Even more critical is the situation of aminoglycosides with high percentages of high-level gentamicin resistance (HLGR) among enterococcal strains worldwide: 45.5% in Brazil [23], 56.9% in Iran [15] and >40% in most of Eu-

ropean countries [22]. The overall pattern of antimicrobial resistances among the enterococci circulating in nosocomial environments makes more challenging the treatment of enterococcal HAIs: daptomycin, linezolid or aminoglycosides are the main alternative antibiotics although various levels of resistance to these drugs have already been described.

Staphylococcus aureus

S. aureus is a Gram-positive bacterium, normally present on human skin but it is also an opportunistic pathogen able to cause a wide range of mild to life-threatening infections. For many years, penicillin has been used successfully to fight infections caused by *Staphylococcus* species but, since its first administration, resistances emerged rapidly within *S. aureus* in 1948 thanks to the synthesis of the penicillinases enzymes [8]. The production of penicillinases, conferring a significant advantage, became a common finding among *S. aureus* isolated both within healthcare facilities and within the community: over few years the rate of β-lactamase-production among *Staphylococci* increased more than 80% [7, 24]. In the 1950s, hospital-isolated *S. aureus* started showing resistance also to other antimicrobial classes (including aminoglycosides, macrolides and tetracyclines); it led to the introduction of new molecules such as methicillin, cloxacillin and flucloxacillin [8]. However, as happened to penicillin, also resistance to methicillin emerged easily among some *S. aureus* strains isolated in England in 1961. These findings represented the appearance of a new notable threat for public health: the methicillin-resistant *S. aureus* (MRSA). The molecular mechanism conferring the ability of evading the activity of methicillin consists in the acquisition of staphylococcal cassette chromosome *mec* (SCC*mec*) elements, which include genes encoding variant penicillin-binding proteins with low affinity for the drug [25]. The prevalence of MRSA infections varies significantly around the world. In the United States, CDC estimates that the number of deaths consequent to invasive MRSA infection in 2011 was higher than 11,000 [19]. In Europe, the proportions of methicillin-resistant among invasive *S. aureus* isolated range from 0% (Iceland) to 57.2% (Romania). Even though the positive trend of MRSA rates reported for decades in literature seems to have finally stopped in Europe, in 2015 several European countries still have MRSA percentages higher than 25% (including Italy, Spain and Greece) [26]. MRSA detection is crucial in order to follow specific measures of control and to avoid inappropriate empirical treatment because MRSA strains usually present also reduced susceptibility to other classes of antibiotics (such as aminoglycosides, macrolides and tetracyclines) [9, 27, 28]. However, these bacteria are not confined in hospital settings as they spread also in community during the past two decades and infections caused by community-acquired MRSA (CA-MRSA) in young previously healthy persons have been increasingly reported, in particular in North America where CA-MRSA were first noticed [29]. The proportion of CA-MRSA among MRSA infected patients varies geographically but the average prevalence reported in recent studies is in the range 30-39% [30, 31]. Many of these CA-MRSA strains carry genes encoding the Panton Valentine Leukocidin (PVL) toxin and, even though the most common infections due to PVL-producing MRSA are mild skin and soft tissue infections, the expression of PVL toxin can lead to sepsis, severe necrotizing fasciitis and haemorrhagic respiratory infection [8, 32]. The treatment of choice for MRSA infection in most cases consists in the class of glycopeptides: vancomycin and teicoplanin. Inevitably, the prolonged use of these antibiotics over years exerted a selective pressure on *S. aureus* inducing some strains to become intermediate-susceptible to vancomycin, with a minimal inhibitory concentration (MIC) of 4-8 μg/mL (vancomycin intermediate *S. aureus* [VISA]) and other strains to become vancomycin-resistant *S. aureus* (VRSA), with a MIC≥16 μg/mL. The VISA isolates with reduced susceptibility to the other glycopeptide, teicoplanin, have been detected and defined glycopeptide-intermediate *S. aureus* [7, 15]. Fortunately, high level glycopeptide resistance is still rare in *S. aureus* and limited to strains that acquired the gene *vanA* from enterococci but, worryingly, an increasing trend of vancomycin MIC in MRSA isolated has been reported by many authors [33]. This phenomenon, known with the term "MIC creep", indicates that the reduced vancomycin-susceptibility is now emerging in *S. aureus* but VRSA remain still rare with only few clinical cases reported in several countries [15]. The rise of VISA is of particular concern because it implies the need for careful

epidemiological surveillance and the search of alternative treatments. Several antibiotics active *in vitro* against VISA isolates are already available: daptomycin, the oxazolidinones linezolid and tedizolid that remains still highly active on *S. aureus* (although mechanisms of resistance relating changes in the 23S rRNA have been shown *in vitro*) and three lipoglycopeptides approved for Gram positive infections (dalbavancin, telavancin and oritavancin) [15, 34]. Finally, the treatment of CA-MRSA infections can rely on a high number of alternative molecules (including clindamycin, fluoroquinolones, gentamicin and tetracyclines) because, unlike most nosocomial-acquired MRSA strains, CA-MRSA strains have not developed relevant multidrug-resistance yet [8].

Klebsiella pneumoniae

The genus *Klebsiella,* member of the Enterobacteriaceae family, includes some of the main pathogens associated with infections acquired in healthcare settings. These infections, characterized by severe morbidity and mortality, include pneumonia, sepsis and urinary tract infections. Among the *Klebsiella* species the most often detected as causative agent of community and hospital-associated infections is represented by the Gram negative bacillus *K. pneumoniae* [7]. This species can rely on several virulence factors, such as its capsule and its fimbrial adhesins, but the most important is the ability of rapidly accumulating multidrug resistance determinants [35]. Indeed, in recent years, many authors reported a marked increasing trend of drug-resistance among *K. pneumoniae* strains. The main routes to resistance to beta-lactams acquired by *K. pneumoniae* consist in pore protein mutations (leading to reduction in outer membrane permeability) and in the synthesis of a large variety of β-lactamase enzymes [7, 36, 37]. Extended spectrum ß-lactamases (ESBLs) were first detected in *K. pneumoniae* strains isolated in Germany in 1983, since then ESBLs-producing *K. pneumoniae* have been reported worldwide. An international prospective study conducted in seven countries across the world between 1996 and 1997, reported that 30.8% of nosocomial invasive *K. pneumoniae* infections were attributable to ESBL-producing strains [38]. In 2001 a study merged data from the networks of resistance surveillance in European ICUs for the years 1990–1999, the results highlighted that the prevalence of ESBL-producing *K. pneumoniae* was not uniform among European countries [39]. The highest prevalence (73%) was observed in *K. pneumoniae* isolated in Turkish ICUs; also Portugal (34%), France (36%) and Russia (33%) presented high rates of resistance while Belgium, Germany, Spain and Sweden showed only a 1-4% prevalence of ESBL producing *Klebsiella* in ICU isolates [39, 40]. In 2013 the CDC estimated the number of infections due to ESBL-positive *Klebsiella* about 17,000 cases with approximately 1,100 deaths attributable to these strains [19]. ESBL are active on penicillins, first-, second- and third- generation cephalosporins but aminoglycosides, fluoroquinolones and carbapenems have been successfully administrated for years to fight *K. pneumoniae* infections, nevertheless the extensive use of these drugs inevitably posed a selective pressure. Therefore, recent data of antimicrobial resistance surveillance show that in most European countries the proportion of aminoglycosides- and fluoroquinolones-resistance among invasive *K. pneumoniae* isolates is >25% [26]. Even the carbapenem group antibiotics, which were considered the drugs of choice for ESBL-producing *K. pneumoniae* infections, often demonstrate only a reduced activity on *Klebsiella* because microorganisms have developed an efficient carbapenem resistance mechanism based on the activity of several enzymes, known as carbapenemases. Carbapenem-resistant *Klebsiellla pneumoniae* (CRKP) is the most frequent and clinically relevant carbapenemase-producing species among the Enterobacteriaceae. A new enzyme, named New Delhi metallo-β-lactamase-1 (NDM-1) belonging to the metallo-β-lactamases, has recently been found in *Klebsiella*. This "super enzyme" differs from other carbapenemases (such as Verona integron-encoded VIM-1/VIM-2) in its structure and affinity to β-lactams, moreover the gene encoding NDM-1 is often allocated in plasmids carrying also other antibiotic-resistance determinants [41]. The worldwide emergence of NDM-1 has furtherly increased the percentages of carbapenem-resistant *K. pneumoniae* (CRKP) [42]. A large study from 42 centres in the USA reported that CRKP accounted for 6.1% of all *K. pneumoniae* isolated [36, 43]. Similar results have been produced by the European Antimicrobial Resistance Surveillance Network (EARS-Net) demonstrating that, in Europe, the population-weighted mean percentage of carbapenem resistance among *Kleb-*

siella is 8%, although the majority of countries had percentages <5% while only three countries reported excessively high carbapenem resistance rates: Romania (24.7%), Italy (33.5%) and Greece (61.9%) [26]. The rapid spread of CRKP in these countries is noteworthy because only few alternative molecules retaining activity on CRKP are currently available (colistin, tigecycline, fosfomycin, gentamicin, amikacin and ceftazidime/avibactam) [26, 44, 45].

Acinetobacter baumannii

Even though most species belonging to the *Acinetobacter* genus are ubiquitous bacteria with low pathogenicity, some *Acinetobacter* species are frequent causes of HAI as they easily spread into hospital environment and can be isolated from skin of patients and hospital staff [7, 46]. In recent years, they have been often detected as causative agents of a vast array of nosocomial infections (including urinary tract infections and ventilator-associated infections) particularly in highly vulnerable ICU patients, intubated or burn patients or those carrying intravenous lines and catheters; while community-acquired infections have been less commonly reported [47]. Among Acinetobacter species the most important nosocomial pathogen is by far *A. baumannii*, an aerobic Gram-negative bacillus, which has a relevantly high ability to survive for prolonged periods in nosocomial environment and on human surfaces, that may explain the frequent cross contamination among hospitalized patients [7]. The clinical relevance of *A. baumannii* as nosocomial pathogen increased in the 1980s, with the emergence of strains developing the ability to evade the action of many antimicrobial molecules, including carbapenems, which were used as the drug of choice for treating *A. baumannii* infections; therefore, they remain susceptible to relatively few drugs (colistin, meropenem, rifampicin and tigecycline) [48]. Not only carbapenem-resistance has been widely reported in Europe but it is also often found combined with resistance to other antimicrobial groups (in particular to fluoroquinolones and aminoglycosides). This pattern of combined resistance to multiple antibiotics has become of concern in countries of southern and south-eastern Europe, where in 2015 the rates of resistance were higher than 50% (72,6% in Italy; 82,2% in Greece and 87% in Croatia) [26]. *Acinetobacter* represents a public health threat also in USA where it is estimated that about 2% of all HAIs diagnosticated in 2013 were due to *Acinetobacter* with approximately 12,000 cases each year (63% of which were attributable to multi-drug resistant *A. baumanni*) [19]. In Turkey, imipenem susceptibility among *A. baumannii* strains has shown a drastic reduction from 50% to 19% during the period 2007-2010 [46]. In China, a nationwide surveillance program conducted from 2005 to 2014, revealed that the rates of imipenem and meropenem resistance among *Acinetobacter* spp. were 57 and 61%, respectively [49]. These species acquired resistance to a vast spectrum of antibiotics through several mechanisms: mutations in ribosomal proteins confer resistance to aminoglycosides; upregulation of efflux systems can explain reduced susceptibility to fluoroquinolones and tigecycline; while resistance to carbapenems is often due to the acquisition of plasmid-mediated genes encoding for metallo-beta-lactamase (MBL) and extended spectrum beta-lactamase (ESBL) enzymes [26, 50, 51]. The most commonly found ESBLs in *A. baumannii* are the PER-, GES- and VEB-type β-lactamases that confer resistance to expanded-spectrum cephalosporins while other ESBLs such as TEM-, SHV- and CTX-M-type, known to be widespread among Enterobacteriaceae, have been rarely identified in *A. baumannii* [52]. This bacterium is also able to produce several metallo-β-lactamases (MBLs), including the recently identified NDM-1. MBLs inactive carbapenems and other β-lactams (except monobactams) and are not inhibited by the β-lactamase inhibitors such as clavulanic acid or tazobactam [52]. However, the high resistance to carbapenems among *A. baumannii* isolates can be explained by the combination of several mechanisms: the synthesis of carbapenem-hydrolysing β-lactamases, the mutational loss of outer membrane proteins and the overexpression of efflux systems [50, 52]. The spread of carbapenem-resistant *A. baumannii* in hospitals has drastically restricted the reliable options for effective antibiotic treatment of *A. baumannii* infections. Currently, colistin alone or in combination with other antimicrobials, including ampicillin-sulbactam, rifampin, tigecycline and carbapenems, is considered the mainstay for infections caused by multidrug resistant (MDR) *A. baumannii*, in particular for patients

who are already under carbapenem therapy [47, 53, 54]. Nevertheless, the return to the use of colistin led bacteria to develop mechanisms of resistance also to colistin (such as mutations of lipopolysaccharide outer membrane) thus the first cases of colistin-resistant *A. baumannii* have already been reported worldwide [54].

Pseudomonas aeruginosa

P. aeruginosa is a Gram-negative, facultative anaerobe opportunistic pathogen, that is present in the normal gut microbiota. Although the rates of *P. aeruginosa* carriers in the general population are not extremely high, this pathogen is widely detected in healthcare settings, being a leading cause of life-threatening HAI worldwide, in particular for immunocompromised patients admitted in ICUs [7]. *P. aeruginosa* has an intrinsic tolerance to many disinfectants and antimicrobial agents but also the ability to acquire adaptive resistance after drug administration, because of the production of efflux pumps and antibiotic-inactivating enzymes (including ESBLs, carbapenemases and metallo-β-lactamases) and the reduced outer membrane porin permeability [7, 55]. Carbapenems are still considered the antimicrobial class of choice to fight *P. aeruginosa* infections, thus the emergence of MDR strains with reduced susceptibility to carbapenems represent a serious concern as it severely compromises the efficacy of this treatment [55]. According to the antimicrobial resistance surveillance data of 2015, high rates of carbapenem resistance are common among invasive *P. aeruginosa* isolated across Europe, with national percentages ranging between <5% (in the U.K. and in The Netherlands) and 66.3% (in Romania). Overall, the mean European percentage for carbapenem resistance was 17.8 % in 2015 [26]. Even more alarming is the fact that in most of the European countries >10% of the *P. aeruginosa* isolates present a combined resistance to three or more antimicrobial groups tested under EARS-Net surveillance (aminoglycosides, carbapenems, ceftazidime, fluoroquinolones, piperacillin and tazobactam) [26]. In the U.S. approximately 13% of all the *Pseudomonas* HAIs are due to these multidrug-resistant strains, that means 6,700 infections each year [19]. Several ESBLs (PER-1; VEB-1 and GES enzymes) are often detected in *Pseudomonas* strains conferring resistance to expanded-spectrum cephalosporins. AmpC is an intrinsic ß-lactamase, expressed at low levels in standard conditions but sub-inhibitory concentrations of certain β-lactams can induce AmpC-synthesis leading to the overexpression of this cephalosporinase. This phenomenon results in the selection of *P. aeruginosa* strains resistant to ceftazidime, piperacillin and ticarcillin [52]. Even though *Pseudomonas* produces carbapenemases (mainly MBLs) its routes to resistance to carbapenems seems to be more related to porin deficiency, which causes a reduced uptake of antimicrobial molecules [52]. All these mechanisms explain the success of these bacteria in causing HAI, indeed such infections have been well described worldwide. In Kosovo, the rate of resistance against imipenem and meropenem among the *P. aeruginosa* collected in 2015 was 37.7% and 36%, respectively [55]. A retrospective study conducted in Italy during the period 2007-2010 reported that 20% of all the *P. aeruginosa* isolated were multi-drug resistant [56]. A recent meta-analysis on patients with pneumonia in China revealed that the resistance rates to cefoperazone, imipenem and meropenem among *P. aeruginosa* causing hospital-acquired pneumonia were 50%, 22.9% and 35.7%, respectively [57]. In the U.S. a large study based on data from a nationally representative sample of microbiology laboratories, reported that the prevalence of multidrug-resistance among *P. aeruginosa* causing pneumonia was 22%, that is a value nearly 15-fold higher than carbapenem-resistant Enterobacteriaceae causing the same type of infection [58]. Also in Taiwan, the overall prevalence of carbapenem-resistant *P. aeuruginosa* is high, approximately 10.2% [59]. On the basis of such studies, reporting only scarce effectiveness of most antibiotics against *Pseudomonas*, except for some new molecules such as ceftolozane/tazobactam, the return to colistin represents a reliable option of treatment. Therefore, despite the risk of colistin adverse effects (nephrotoxicity and neurotoxicity), its use has been reconsidered in particular for the synergistic activity with other antibiotics (in order to avoid the emergence of resistance during monotherapy) [60].

Enterobacter spp.

Enterobacter species are emerging as common Gram-negative pathogens, being often detected as causative agents of HAIs, in particular septicaemia, respiratory and urinary tracts infections

[35]. Currently, *Enterobacter* spp. are the eighth most common cause of HAI in the U.S., although they have been also implicated in community-acquired infections [61-63]. *Enterobacter* species have shown the ability to develop antimicrobial resistance thanks to the hyperproduction of a chromosomally encoded AmpC β-lactamase, which can hydrolyze broad-spectrum cephalosporins and penicillins [64, 65]. As explained above, the hyperproduction of AmpC can be induced by the exposure to beta-lactams and carbapenems, therefore previously sensible *Enterobacter* isolates often become resistant to cephalosporins during therapy and this change is associated with increased mortality [62, 64]. The prevalence of multidrug-resistance in the U.S. has proven to differ among species: 4.9% in *E. aerogenes* whilst 9.5% in *E. cloacae* [66]. A surveillance study conducted in Saudi Arabia during 2000-2006 reported that, on a total of 1,394 Enterobacter isolates, the resistance rate increased from 9 to 17.9% for ceftriaxone; from 8.3% to 17.4% for ciprofloxacin [67]. Many species belonging to Enterobacteriaceae family produce a wide array of ESBLs and carbapenemases that reduce their pattern of antimicrobial susceptibility but such enzymes were supposed to be only rarely detected in *Enterobacter* spp. However, in recent years the clonal dissemination of carbapenem-resistant *Enterobacter aerogenes* (CREA) and their ability to synthetize both metallo-(IMP-8, NDM-1, VIM-1) and non-metallo-(KPC-2) β-lactamases have been widely reported [68]. An outbreak of ESBL-producing *Enterobacter cloacae* has been reported in a neonatal intensive care unit in Spain, with 14 newborns infected or colonized by CTX-M-producing *E. cloacae* strains [69]. Moreover, a recent study reported that during a 1-year period several strains of *E. cloacae* producing ESBLs and carbapenemases have been isolated in a Spanish hospital from patients with critical underlying conditions [65]. In Brazil, 20% of *Enterobacter spp* isolated in patients with bacteremia were ESBLs-producers [70]. In Japan only 6% of the Enterobacter strains isolated produce ESBLs and all these bacteria were resistant to ampicillin and piperacillin and most of them were also resistant to piperacillin-tazobactam and cefoxitin [71]. In 2011, in a neurosurgical ward of a Chinese teaching hospital, all the carbapenem-resistant *Enterobacter aerogenes* (CREA) isolated were non-susceptible to cefotaxime, ceftazidime, cefoxitin, ertapenem, imipenem or meropenem and were proved to carry the gene encoding *Klebsiella pneumoniae* carbapenemase-2 (KPC-2) [72]. In another study in China the 72.7% of the carbapenem-resistant *E. cloacae* isolated were able to produce also the New Delhi metallo-β-lactamase 1 (NDM-1) [73]. Only few antimicrobial compounds still remain effective against the multidrug resistant *Enterobacter spp*, as these bacteria can rapidly develop resistance during treatment. Therefore, the treatment options are often limited to colistin and tigecycline [35].

CONCLUSIONS

The emergence of multidrug-resistance bacteria represents a serious public health threat because their multiple patterns of resistance make the choice of an effective treatment even more challenging. Fortunately, although the number of antimicrobial compounds retaining activity on these pathogens has gradually decreased over the years, the recent development of new molecules with anti-Gram-positive (dalbavancin and telavancin) and anti-Gram-negative bacteria (ceftolozane/tazobactam and ceftazidime/avibactam) activity contributes to increase the treatment options for these infections. Among the MDR bacterial species the group of ESKAPE pathogens *(Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa and Enterobacter spp*.) plays a clinically relevant role, in particular in the aetiology of life-threatening HAIs. In this review we report many studies showing that the burden of these infections has drastically risen worldwide over last decades. ESKAPE pathogens have developed a wide array of strategies to evade the killing activity of the most commonly administrated antibiotics, including the horizontal gene transfer among species, which furtherly enhances the spread of antibiotic-resistances. The only weapons we have to front the rise of these multi-resistant bacteria in the near future are the continuous finding of new active antimicrobial molecules, the careful epidemiological surveillance and the implementation of appropriate infection control programs.

Conflict of interest
The authors declare no conflict of interest.

REFERENCES

[1] Xia J., Gao J., Tang W. Nosocomial infection and its molecular mechanisms of antibiotic resistance. *Biosci. Trends* 10, 14-21, 2016.
[2] Esposito S., Leone S., Noviello S., Ianniello F., Fiore M. Antibiotic resistance in long-term care facilities. *New Microbiol*. 30, 326-331, 2007.
[3] Klevens R.M., Edwards J.R., Richards C.L. Jr., et al. Estimating health care-associated infections and deaths in U.S. hospitals, 2002. *Public Health Rep*. 122, 160-166, 2007.
[4] Magill S.S., Edwards J.R., Bamberg W., et al. Multistate point-prevalence survey of health care-associated infections. *N. Engl. J. Med*. 370, 1198-1208, 2014.
[5] European Centre for Disease Prevention and Control. Point prevalence survey of healthcare-associated infections and antimicrobial use in European acute care hospitals. Stockholm: ECDC; 2013.
[6] Allegranzi B., Bagheri Nejad S., Combescure C., et al. Burden of endemic health-care-associated infection in developing countries: systematic review and meta-analysis. *Lancet* 377, 228-241, 2011.
[7] Santajit S., Indrawattana N. Mechanisms of Antimicrobial Resistance in ESKAPE Pathogens. *Biomed. Res. Int*. 2016: 2475067, 2016.
[8] Hawkey P.M. Molecular epidemiology of clinically significant antibiotic resistance genes. *Br. J. Pharmacol*. 153, S406-S413, 2008.
[9] Nikaido H. Multidrug resistance in bacteria. *Annu. Rev. Biochem*. 78, 119-146, 2009.
[10] Reale M., Strazzulla A., Quirino A., et al. Patterns of multi-drug resistant bacteria at first culture from patients admitted to a third level University hospital in Calabria from 2011 to 2014: implications for empirical therapy and infection control. I*nfez. Med*. 25, 98-107, 2017.
[11] Esposito S., Gioia R., De Simone G., et al. Bacterial Epidemiology and Antimicrobial Resistance in the Surgery Wards of a Large Teaching Hospital in Southern Italy. *Mediterr. J. Hematol. Infect. Dis*. 7: e2015040, 2015.
[12] Esposito S., Pascale R., Esposito I., et al. Epidemiology and antibiotic resistance in a large Italian teaching hospital. *J. Chemother*. 27, 163-166, 2015.
[13] Arias C.A., Murray B.E. The rise of the Enterococcus: beyond vancomycin resistance. *Nat. Rev. Microbiol*. 10, 266-278, 2012.
[14] Saeidi S., Mirnejad R., Masoumi Zavaryani S., Rostamzadeh S. Molecular epidemiology and antibiotic resistance patterns of Enterococcus faecalis isolates from hospitals in Tehran. *Infez. Med*. 25, 116-122, 2017.
[15] Mirnejad R., Sajjadi N., Masoumi Zavaryani S., Piranfar V., Hajihosseini M., Roshanfekr M. Identification of aminoglycoside resistance genes by Triplex PCR in Enterococcus spp. isolated from ICUs. *Infez. Med*. 24, 222-229, 2016.
[16] Top J., Willems R., van der Velden S., Asbroek M., Bonten M. Emergence of clonal complex 17 Enterococcus faecium in The Netherlands. *J. Clin. Microbiol*. 46, 214-219, 2008.
[17] Munita J.M., Bayer A.S., Arias C.A. Evolving resistance among Gram-positive pathogens. *Clin. Infect. Dis*. 61, S48-S57, 2015.
[18] Ramsey A.M., Zilberberg M.D. Secular trends of hospitalization with vancomycin-resistant enterococcus infection in the United States, 2000-2006. *Infect. Control Hosp. Epidemiol*. 30, 184-186, 2009.
[19] Centers for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States, 2013. Centers for Disease Control and Prevention, Atlanta, GA. Retrieved from http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Last accessed November 25th, 2017.
[20] Panesso D., Reyes J., Rincòn S., et al. Molecular epidemiology of vancomycin-resistant Enterococcus faecium: a prospective, multicenter study in South American hospitals. *J. Clin. Microbiol*. 48, 1562-1569, 2010.
[21] Thongkoom P., Kanjanahareutai S., Chantrakooptungool S., Rahule S. Vancomycin-resistant enterococci (VRE) isolates isolated in Rajavithi Hospital between 1999 and 2009. *J. Med. Assoc. Thai*. 95, S7-S15, 2012.
[22] The European Antimicrobial Resistance Surveillance System. EARSS results [Database on the internet]. [Cited 20.09.2017]. Retrieved from http://www.rivm.nl/earss/result/ Last accessed November 25th, 2017.
[23] Vigani A.G., Oliveira A.M., Bratfich O.J., Stucchi R.S., Moretti M.L. Clinical, epidemiological, and microbiological characteristics of bacteremia caused by high-level gentamicin-resistant Enterococcus faecalis. *Braz. J. Med. Biol. Res*. 41, 890-895, 2008.
[24] Appelbaum P.C. Microbiology of antibiotic resistance in Staphylococcus aureus. *Clin. Infect. Dis*. 45, S165-S170, 2007.
[25] Paterson G.K., Morgan F.J., Harrison E.M., et al. Prevalence and characterization of human mecC methicillin-resistant Staphylococcus aureus isolates in England. *J. Antimicrob. Chemother*. 69, 907-910, 2014.
[26] European Centre for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe 2015. Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net). Stockholm: ECDC; 2017.
[27] Tekin A., Dal T., Deveci O., Tekin R., Atmaca S., Dayan S. Assessment of methicillin and clindamycin resistance patterns in Staphylococcus aureus isolated from a tertiary hospital in Turkey. *Infez. Med*. 21, 111-116, 2013.
[28] Callejo-Torre F., Eiros Bouza J.M., Olaechea Astigarraga P., et al. Risk factors for methicillin-resistant Staphylococcus aureus colonisation or infection in intensive care units and their reliability for predicting MRSA on ICU admission. *Infez. Med*. 24, 201-209, 2016.

[29] Maltezou H.C., Giamarellou H. Community-acquired methicillin-resistant Staphylococcus aureus infections. *Int. J. Antimicrob. Agents*. 27, 87-96, 2006.
[30] Li S., Li J., Qiao Y., Ning X., Zeng T., Shen X. Prevalence and invasiveness of community-acquired methicillin-resistant Staphylococcus aureus: a meta-analysis. *Indian. J. Pathol. Microbiol*. 57, 418-422, 2014.
[31] Salgado C.D., Farr B.M., Calfee D.P. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clin. Infect. Dis*. 36, 131-9, 2003.
[32] Esposito S., Bassetti M., Borrè S., et al. Diagnosis and management of skin and soft-tissue infections (SSTI): a literature review and consensus statement on behalf of the Italian Society of Infectious Diseases and International Society of Chemotherapy. *J. Chemother*. 23, 251-262, 2011.
[33] Aschbacher R., Pichon B., Wootton M., et al. Molecular epidemiology of methicillin-resistant from bacteraemia in northern Italy. *Infez. Med*. 20, 256-264, 2012.
[34] Leone S., Noviello S., Boccia G., De Caro F., Esposito S. Methicillin-resistant *Staphylococcus aureus* infections: role of daptomycin/β-lactams combination. *Infez. Med*. 23, 99-104, 2015.
[35] Pendleton J.N., Gorman S.P., Gilmore B.F. Clinical relevance of the ESKAPE pathogens. *Expert Rev. Anti Infect. Ther*. 11, 297-308, 2013.
[36] Cetinkol Y., Yildirim A.A., Telli M., Calgin M.K. The investigation of oxacillinase/metallo-beta-lactamase genes and clonal analysis in carbapenem-resistant *Klebsiella pneumoniae*. *Infez. Med*. 24, 48-53, 2016.
[37] Calitri C., Scolfaro C., Colombo S., et al. Extended-Spectrum Beta Lactamase-producing Enterobacteriaceae among the pediatric population: who is at risk and why? Results from a single-centre prospective study. *Infez. Med*. 24, 318-325, 2016.
[38] Paterson D.L., Ko W.C., Von Gottberg A., et al. International prospective study of *Klebsiella pneumoniae* bacteremia: implications of extended-spectrum beta-lactamase production in nosocomial Infections. *Ann. Intern. Med*. 140, 26-32, 2004.
[39] Hanberger H., Diekema D., Fluit A., et al. Surveillance of antibiotic resistance in European ICUs. *J. Hosp. Infect*. 48, 161-176, 2001.
[40] Giamarellou H. Multidrug resistance in Gram-negative bacteria that produce extended-spectrum beta-lactamases (ESBLs). *Clin. Microbiol. Infect*. 11, 1-16, 2005.
[41] Yong D., Toleman M.A., Giske C.G., et al. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in Klebsiella pneumoniae sequence type 14 from India. *Antimicrob. Agents Chemother*. 53, 5046-5054, 2009.
[42] Pittalis S., Ferarro F., Puro V. [NDM-1: the superbug?]. *Infez. Med*. 19, 224-234, 2011.
[43] Kaiser D.M., Castanheira M., Jones R.N., Tenover F., Lynfield R. Trends in *Klebsiella pneumoniae* carbapenemase positive *K. pneumoniae* in US hospitals: report from the 2007-2009 SENTRY Antimicrobial Surveillance Program. *Diagn. Microbiol. Infect. Dis*. 76, 356-360, 2013.
[44] De Rosa F.G., Corcione S., Raviolo S., et al. Candidemia, and infections by *Clostridium difficile* and carbapenemase-producing Enterobacteriaceae: new enteropathogenetic opportunistic syndromes? *Infez. Med*. 23, 105-116, 2015.
[45] van Duin D., Bonomo R.A. Ceftazidime/Avibactam and Ceftolozane/Tazobactam: Second-generation β-Lactam/β-Lactamase Inhibitor Combinations. *Clin. Infect. Dis*. 63, 234-241, 2016.
[46] Deveci Ö., Dal T., Tekin R., Bozkurt F., Tekin A., Dayan S. Carbapenem resistance in *Acinetobacter baumannii*: where is it heading? *Infez. Med*. 21, 211-215, 2013.
[47] Metan G., Pala Ç., Kaynar L., Cevahir F., Alp E. A nightmare for haematology clinics: extensively drug-resistant (XDR) Acinetobacter baumannnii. *Infez. Med*. 22, 277-282, 2014.
[48] Buccoliero G., Morelli E., Lonero G., Romanelli C., Resta F., Pisconti S. Rapid spread of multiresistant Acinetobacter baumannii isolates in intensive care units (ICUs) and in vitro activity of colistin and tigecycline. *Infez. Med*. 20, 296-298, 2012.
[49] Wang F., Zhu D., Hu F., et al. CHINET 2012 surveillance of bacterial resistance in China. *China J. Infect. Chemother*. 13, 321-330, 2013.
[50] Owlia P., Azimi L., Gholami A., Asghari B., Lari AR. ESBL- and MBL-mediated resistance in *Acinetobacter baumannii*: a global threat to burn patients. *Infez. Med*. 20, 182-7, 2012.
[51] Antunes L.C., Visca P., Towner K.J. Acinetobacter baumannii: evolution of a global pathogen. *Pathog. Dis*. 71, 292-301, 2014.
[52] Potron A., Poirel L., Nordmann P. Emerging broad-spectrum resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii*: Mechanisms and epidemiology. *Int. J. Antimicrob. Agents* 45, 568-585, 2015.
[53] Ece G., Samlioglu P., Atalay S., Kose S. Evaluation of the in vitro colistin susceptibility of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* strains at a tertiary care centre in Western Turkey. *Infez. Med*. 22, 36-40, 2014.
[54] Qureshi Z.A., Hittle L.E., O'Hara J.A., et al. Colistin-resistant *Acinetobacter baumannii*: beyond carbapenem resistance. *Clin. Infect. Dis*. 60, 1295-303, 2015.
[55] Lila G., Mulliqi-Osmani G., Bajrami R., Kurti A., Azizi E., Raka L. The prevalence and resistance patterns of *Pseudomonas aeruginosa* in a tertiary care hospital in Kosovo. *Infez. Med*. 25, 21-26, 2017.
[56] De Francesco M.A., Ravizzola G., Peroni L., Bonfanti C., Manca N. Prevalence of multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* in

an Italian hospital. *J. Infect. Public Health* 6, 179-85, 2013.
[57] Ding C., Yang Z., Wang J., et al. Prevalence of *Pseudomonas aeruginosa* and antimicrobial-resistant *Pseudomonas aeruginosa* in patients with pneumonia in mainland China: a systematic review and meta-analysis. *Int. J. Infect. Dis.* 49, 119-128, 2016.
[58] Zilberberg M.D., Shorr A.F. Prevalence of multidrug-resistant *Pseudomonas aeruginosa* and carbapenem-resistant Enterobacteriaceae among specimens from hospitalized patients with pneumonia and bloodstream infections in the United States from 2000 to 2009. *J. Hosp. Med*. 8, 559-563, 2013.
[59] Lin K.Y., Lauderdale T.L., Wang J.T., Chang S.C. Carbapenem-resistant *Pseudomonas aeruginosa* in Taiwan: Prevalence, risk factors, and impact on outcome of infections. *J. Microbiol. Immunol. Infect*. 49, 52-59, 2016.
[60] Ly N.S., Bulitta J.B., Rao G.G., et al. Colistin and doripenem combinations against *Pseudomonas aeruginosa*: profiling the time course of synergistic killing and prevention of resistance. *J. Antimicrob. Chemother*. 70, 1434-1442, 2015.
[61] Sievert D.M., Ricks P., Edwards J.R., et al. Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2009-2010. *Infect. Control Hosp. Epidemiol.* 34, 1-14, 2013.
[62] Huh K., Kang C.I., Kim J., et al. Risk factors and treatment outcomes of bloodstream infection caused by extended-spectrum cephalosporin-resistant Enterobacter species in adults with cancer. *Diagn. Microbiol. Infect. Dis*. 78, 172-177, 2014.
[63] Tedesco Maiullari R.M., Guevara A. [Enterobacteria producers of extended-spectrum beta-lactamase in a hospital from Venezuela]. *Infez. Med*. 20, 93-99, 2012.
[64] Schwaber M.J., Graham C.S., Sands B.E., Gold H.S., Carmeli Y. Treatment with a broad-spectrum cephalosporin versus piperacillin-tazobactam and the risk for isolation of broad-spectrum cephalosporin-resistant Enterobacter species. *Antimicrob. Agents Chemother*. 47, 1882-1886, 2003.
[65] Fernández J., Montero I., Martínez Ó., et al. Dissemination of multiresistant Enterobacter cloacae isolates producing OXA-48 and CTX-M-15 in a Spanish hospital. *Int. J. Antimicrob. Agents*. 46, 469-474, 2015.
[66] DiPersio J.R., Dowzicky M.J. Regional variations in multidrug resistance among Enterobacteriaceae in the USA and comparative activity of tigecycline, a new glycylcycline antimicrobial. *Int. J. Antimicrob. Agents*. 29, 518-527, 2007.
[67] Al-Tawfiq J.A., Antony A., Abed M.S. Antimicrobial resistance rates of Enterobacter spp.: a seven-year surveillance study. *Med. Princ. Pract*. 18, 100-104, 2009.
[68] Liu C., Qin S., Xu H., et al. New Delhi Metallo-β-Lactamase 1(NDM-1), the Dominant Carbapenemase Detected in Carbapenem-Resistant *Enterobacter cloacae* from Henan Province, China. *PLoS One*, 10: e0140726, 2015.
[69] Oteo J., Cercenado E., Vindel A., et al. Outbreak of multidrug-resistant CTX-M-15-producing Enterobacter cloacae in a neonatal intensive care unit. *J. Med. Microbiol*. 62, 571-575, 2013.
[70] Nogueira Kda S., Paganini M.C., Conte A., et al. Emergence of extended-spectrum β-lactamase producing *Enterobacter* spp. in patients with bacteremia in a tertiary hospital in southern Brazil. *Enferm. Infecc. Microbiol. Clin*. 32, 87-92, 2014.
[71] Kanamori H., Yano H., Hirakata Y., et al. Molecular characteristics of extended-spectrum beta-lactamases and qnr determinants in Enterobacter species from Japan. *PLoS One*, 7: e37967, 2012.
[72] Qin X., Yang Y., Hu F., Zhu D. Hospital clonal dissemination of *Enterobacter aerogenes* producing carbapenemase KPC-2 in a Chinese teaching hospital. *J. Med. Microbiol.* 63, 222-228, 2014.
[73] Qin S., Fu Y., Zhang Q., et al. High incidence and endemic spread of NDM-1-positive Enterobacteriaceae in Henan Province, China. *Antimicrob. Agents Chemother*. 58, 4275-4282, 2014.