Current trends in epidemiology and antimicrobial resistance in intensive care units

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Abstract: Intensive care units (ICUs) have a unique environment within the hospital setting. The fragility of the patients facilitates the presence of hospital-acquired infections (HAIs). To minimize the impact of HAIs, a series of antibiotic stewardship strategies including mixing, cycling and combination therapies have been developed. Nonetheless, the impact of antibiotic resistance in HAIs in ICUs remains extremely high. Most HAIs are caused by multidrug-resistant microorganisms, originating from contaminated ICUs surfaces or devices or from patients admitted for infectious processes. Moreover, antibiotic resistance may remain occult and be silently introduced into the ICU by a commensal member of the microbiomes of patients or health care personnel and thereafter transmitted to a pathogenic microorganism already residing in the ICU. The pathogenic microorganisms most frequently residing in ICUs include *Escherichia coli* and members of the so-called ESKAPE group (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Enterobacter spp.*). These microorganisms show resistance to most of the antibacterial agents commonly used in ICUs and have been associated with the presence of specific mechanisms of chromosomal or transferable resistance. Furthermore, when more than one antibiotic-resistant determinant is encoded within the same genetic structure lead to the phenomena of co-selection of resistance. In some cases, ESKAPE microorganisms show resistance to all the scheduled antibacterial agents, requiring the use of "last resort" antibacterial agents such as ceftaroline, colistin or tigecycline. Unfortunately, resistance to all these antimicrobial agents has been reported. The current antibiotic resistance observed in ICUs is of great concern and is dramatically advancing to a worrisome end: the return to a pre-antibiotic era in which a series of antibiotic-untreatable pan-resistant microorganisms colonize ICUs causing severe and deadly infections. Urgent actions to firmly control antibiotic use in all environments are essential to put a halt to or mitigate the development of antibiotic resistance, while the development of new treatment alternatives is a research priority.

Keywords: ESKAPE; hospital-acquired infection; antibiotic-resistance

The environment of intensive care units (ICUs)

Despite the relevance of other environments such as veterinary settings, two different environments are classically considered when referring to human infections: community and hospital. Nonetheless, these two environments also have internal differences which produce special "internal micro-environments", in this sense, ICUs are among the most unique internal micro-environment within a hospital

setting. These units attend severe and critically ill patients, requiring very special care because of their fragile situation. In ICUs, the specific situation of the patients attended, such as the extremes of life, underlying diseases, high risk surgeries and the frequent need for invasive devices such as central or peripheral venous catheters, urinary catheters or tracheal tubes, among others, makes them more susceptible to acquiring infections (1). Thus, a report including 231,459 patients from 947 hospitals within the European Union (EU) during the period from 2011–2012 showed that 19.5% of the 11,516 patients admitted to an ICU acquired an infection during their hospital stay (hereafter referred to as hospital-acquired infection or HAI), with this rate decreasing to 5.2% (P<0.0001) among the remaining hospitalized patients (2). Despite a clear trend to a decrease in HAI in the USA, on comparing data from 2016 with those from 2008 (3), the 2016 data from more than 23,000 health care institutions and hospital settings showed that in 1 out of 30 hospitals a HAI was acquired daily (4).

The most recent data from the EU obtained in 2016 focused on infections acquired in ICUs, including 151,709 patients admitted to 1,451 ICUs belonging to 1,159 hospital settings in 15 European countries. It was reported that 12,735 (8.4%) patients admitted to an ICU for more than 2 days acquired at least one HAI (5). These HAIs included 9,569 cases of pneumonia (97% related to patient intubation), 5,579 blood stream infections and 1,395 urinary tract infections (UTIs); although also present, no other acquired infections (such as skin or ocular) were included in the analysis (5). In this study, the so-called "ESKAPE" microorganisms (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter spp.*) were frequently isolated (5).

HAI causes increased morbidity and related treatment costs, which in the EU has been estimated at around 16 million additional days of hospitalization and more than €5.5 billion per year. However, what is more relevant is that the presence of HAIs in ICU environments may be especially life-threatening. In fact, it is estimated that in the EU, HAIs underlie more than 37,000 deaths each year, mostly involving ICU patients (6).

The fight against HAIs, especially in ICUs, has mainly been focused on the presence of preventive measures, most including bacterial barriers such as limited access of patients to potential external sources of contamination or the presence of strict hygiene measures among patients, health care personnel or the ICU itself (7). Furthermore, since the explosive introduction of antimicrobial agents into clinical practice in the 1940's (8), these medicaments have become the main weapon, not only in the battle against infections but also in the prevention of infection through prophylactic use. This latter application of antibacterial agents rapidly extended to especially at-risk populations including ICU patients (9). Nonetheless, the usefulness of the current antibiotic prophylactic schedules is challenged by the acquisition, development and spread of antibiotic resistance (9).

Unfortunately, several microorganisms such as the above-mentioned members of the ESKAPE group, have great facility to acquire or develop antibiotic resistance and enhanced tolerance to disinfectants (10,11). This finding, concomitantly with the commented basal characteristics of the patients, long hospital stays and especially the need to use antibacterial agents in the treatment or prevention of HAIs have resulted in the current high levels of antibacterial resistance related to the ICU environment, contributing to the presence of ICUs outbreaks related to multidrug-resistant pathogenic microorganisms (12,13).

Antimicrobial agents

Natural antibiotics are almost as old as microorganisms. Their natural function is to kill and eliminate the "competence" and/or obtain nutrients by the lysis of neighboring microorganisms. Although the empirical use of molds to avoid infections may be traced to several centuries ago, the first scientific studies of antimicrobial agents' date from 19th century, when the ability of different microorganisms to inhibit the growth of others was observed (14). Thereafter, the first antibacterial agent (the so-called piocianase, produced by *Pseudomonas aeruginosa*) was introduced into clinical practice in 1899 (15), and Tiberio and Duchesne first described penicillin in 1895 and 1897, respectively (16,17). Furthermore, in the first third of the 20th century substances, such as the arsenic derivative salvarsan or sulphamides, were largely used to treat infections (18,19). Nevertheless, it is largely considered that the antibiotic era was heralded in 1928 and officially appeared in the 1940's with Fleming's description of penicillin and its introduction into clinical practice during World War II (7,20).

A variety of antibacterial agents were isolated or synthetized between the 1940's and 1960's. Thereafter, the number of truly different antibacterial agents markedly decreased, with new members of known antibacterial agents presenting higher spectrums or higher levels of activity,

Table 1 Main threats of antibiotic resistance profile and last treatment resorts

Microorganisms	Threats	Last resort
Enterococcus spp.	XDR (Van^R)	Daptomycin, tigecycline
Staphylococcus aureus	XDR	Gly, Ceft, Caz/Avb, Cft/Taz
Klebsiella pneumoniae	XDR (C3G^R, Cbp^R)	Colistin, tigecycline
Acinetobacter baumannii	PDR^R	Colistin, tigecycline
Pseudomonas aeruginosa	PDR^R	Colistin
Enterobacter spp.	XDR	Colistin, tigecycline
Escherichia coli	XDR (C3G^R, Cbp^R)	Colistin

XDR, extremely-drug resistant; PDR, pan-drug resistant; C3G, third generation cephalosporins; Cbp, carbapenems; Gly, glycopeptides; Ceft, ceftaroline; Caz/Avb, ceftazidime plus avibactam; Cft/Taz, ceftolozane plus tazobactam.

but targeting the same target, mainly being developed. While it seemed that infectious diseases had been defeated and had been rendered to oblivion during the first decades of the antibiotic era, the screenplay of history promptly turned around and bacteria presented an unexpected, albeit predictable (21), defensive response: the development of antibacterial resistance.

Nowadays, in ICUs, antibiotic options to treat the main pathogens causing HAI are scarce. In addition, apart from the development of only a few new antibiotics, several pharmaceutical companies have announced that they will no longer invest in the development of new drugs (22). Thus, the presence of HAIs caused by microorganisms highly resistant to all antimicrobials is an emerging reality. In these cases, no standard antibiotic schedule is effective and frequently the use of older and disused antibacterial compounds is considered as the last resort (23). As mentioned above, some old antibiotic compounds such as polymyxins (polymyxin B and E), several aminoglycosides or fosfomycin have been re-introduced as an alternative for the treatment of extended-spectrum beta lactamases (ESBL) and carbapenemases-producing pathogens (24). Meanwhile, a few newly developed agents, such as daptomycin and telavancin, have become options to treat multi-resistant Gram-positive microorganisms (25,26) (*Table 1*).

Antimicrobial resistance

The first reports of antimicrobial resistance are as old as the clinical use of antibacterial agents, having been described prior to the introduction of penicillin to clinical practice (21). Although in the early years of the antibiotic era this phenomenon seemed to be

more a scientific curiosity than a true clinical problem, the subsequent wide use of antibacterial agents led to changes in the structure of bacterial populations, with microorganisms susceptible to antibacterial agents being selectively killed, and those carrying or acquiring specific antibiotic resistance determinants being selected (27). This finding resulted in a continuous increase in the isolation of antibiotic-resistant microorganisms, and at present, bacterial antimicrobial resistance is one of the top-ranked problems challenging the current clinical practices, underlying long-term hospitalizations, increasing infection treatment costs and impacting the survival of infected patients (27,28). Thus, it has been considered that antibiotic resistance worldwide results in more than 700,000 deaths per year (27), and the most pessimist future predictions consider that this number will rise to >10 million deaths per year in the next 30 years (27). Obviously, the impact on ICU patients is especially worrisome. For instance, 40% of ICU patients colonized by carbapenem-resistant *Enterobacteriaceae* have a fatal outcome, *vs.* ~28% of patients colonized by carbapenem-susceptible *Enterobacteriaceae* (29). Furthermore, ICU infections by carbapenem-resistant *Klebsiella pneumoniae* showed a 20% higher adjusted mortality rate than those related to carbapenem-susceptible *Klebsiella pneumoniae* (30). These findings highlight the impact on mortality related to the presence of antibiotic-resistant microorganisms primarily identified as either a cause of colonization or infection. In this line, a meta-analysis including 15 studies accounting for 3,201 *Pseudomonas aeruginosa* from 5 countries showed that infections related to multidrug-resistant *Pseudomonas aeruginosa* resulted in 44.6% deaths *vs.* 24.8% deaths related to infections by

non-multidrug-resistant *Pseudomonas aeruginosa* (31).

Among the reasons underlying this increased mortality it should be mentioned that the high levels of antibiotic resistance strongly contribute to initial inappropriate empiric antibiotic treatments and the subsequent delay in the implementation of adequate treatments (32,33). In fact, on analyzing infections by carbapenem-resistant *Klebsiella pneumoniae* it was considered that a lack of microbiologic eradication at 7 days led to an increase in 30-day mortality (34). In this line, while the impact on bacteremia mortality related to ESBL-producing *Escherichia coli* was more than double that related to non-ESBL *Escherichia coli* (37.5% *vs.* 15.6%; P=0.04), no differences were found when the analysis was limited to patients with an appropriate initial empiric treatment (33).

It is of special concern that in the current interconnected world, the dissemination of microorganisms is as wide and rapid as allow the current travel and trade routes (35). Thus, it is only a question of time for a microorganism carrying a specific antibiotic resistance mechanism, through the microbiota of a patient's relative or member of ICU staff or a clinical device, to reach an ICU and cause infection. Therefore, preventive measures against infection are key to avoid the establishment of these microorganisms or the dissemination of the mechanisms of resistance to other microorganisms present in the ICU.

Development of antibiotic resistance

The development of antibiotic resistance has been related to different factors, but undoubtedly the most important is the antibiotic pressure of the antibiotic itself (27). As above commented factors such as long hospital-stays or the use of antibacterial agents are primary causes of the development of antibiotic-resistant microorganisms in hospital settings. These factors are not modifiable and only a continuous surveillance, leading to early detection of antibiotic-resistant microorganisms, together hygienic measures and antibiotic control strategies may be implemented to minimize its impact on the development and acquisition of antibiotic resistance (see below). Nonetheless, the establishment of some specific measures, such as an active sought for asymptomatic carriers of antibiotic-resistant microorganisms when patients admitted, or preventive measures when patients transferred from other hospital centers may contribute to minimize the most severe risk factor: the silent introduction of antibiotic-resistant determinants. In this sense, undoubtable, the most relevant

antibiotic pressure exerted on microorganisms is derived from the use of antibacterial agents out of hospital settings. Therefore, the uncontrolled access to antibacterial agents, the lack of sanitary education or the pressures exerted over antibiotic-prescribing personnel lead to unnecessary (e.g., treatment of cold or self-limiting diarrhea) or inadequate use of antibacterial agents as well as premature treatment stops or incorrect dosages among other misuses of antibacterial agents. More relevant: worldwide the antibiotic consumption in veterinary settings has been estimated at 63,151 (±1,560) tons in 2010 and is considered to rise to 105,596 (±3,605) tons, by 2030 (increase ~67%) (36); most of these antimicrobial agents being used in healthy animals as growth promoters. When measures to control, regulate and limit the access of antibiotics in community and veterinary settings are truly established and implemented, they have a direct impact on the final antibiotic resistance levels of community pathogens, and thereby contribute to limiting the development and spread of antibiotic-resistant microorganisms (37). On the other hand, over time, nosocomial pathogens are under the pressure of the most potent antibacterial agents which are ideally reserved for use in clinical settings. In this sense, it has been established that in the EU in the period from 2011–2012 the prevalence of hospitalized patients receiving at least one antibiotic during their stay was 35%, increasing to 56.5% among patients admitted to an ICU (37). This finding demonstrates the enhanced antibiotic pressure that is exerted within ICUs and contributes to the selection of hospital-adapted pathogenic microorganisms exhibiting higher levels of antibiotic resistance and highlights the need for strict antibiotic control measures in all hospital settings, especially ICUs.

Spread of antibiotic resistance

The spread of antibiotic resistance within ICUs is of note. This suggests that a specific genetic structure may be selected to enable dissemination within microorganisms due to the bacterial promiscuity, or that a specific bacterial clone may be selected. While the first scenario results in an increase in the number of infections or colonization by different antibiotic-resistant microorganisms, the second one usually results in the presence of a bacterial clone well adapted to the ICU environment; these clones are often especially virulent and some have been named "high risk clones" (12,13). The presence of these "high risk" clones in ICUs has been related to increased mortality (12).

Prevention and control of antibiotic resistance

While control and restriction in the use of antibiotics are the best road to fight against the development and spread of antibiotic-resistant microorganisms, both within and without hospitals settings (38), Jano, the Roman two-faced god, may be the best approach to control antibiotic resistance in ICUs. In the Roman mythology, Jano represents the beginning and the ending, the past and the future. Similarly, the fragile situation of ICU patients often necessitates the use of antibacterial agents even in the absence of confirmation of infection, but this use of antibiotics contributes to exacerbating the risk of infections by ICU-selected multidrug-resistant nosocomial pathogens (30).

In this interphase several approaches of antibiotic use have been developed to contribute to the control and to limit the spread of antibiotic-resistant microorganisms in ICUs (39,40). Thus, several authors have proposed the so-called "mixing", in which the heterogeneity of the antibacterial agents used is maximized in order to minimize the specific pressure exerted by each agent (41). The objective is to avoid the spread of selected antimicrobial-resistant microorganisms by introducing a diversity of treatments (39).

Other authors have proposed a strategy based on different antibiotic cycles in the ICU (the so-called "cycling") with the objective of eliminating selected antibiotic-resistant microorganisms with a cycle change (39). Finally, a third strategy is based on combined therapies to hinder resistance and the inherent fitness costs (40). Nonetheless, the true final effect of these measures is controversial. Thus, while some authors have reported that the use of completely unrelated antibacterial agents in either cycling or mixing strategies addressed to different bacterial targets contributes to the control of different microorganisms, including carbapenem-resistant *Pseudomonas aeruginosa* (42), others have not observed any effect on the control of antibiotic resistance using any of these approaches (38).

The ESKAPE group

The so-called ESKAPE group deserves special attention due to the combination of innate virulence with higher levels of antibiotic resistance (11,43). These microorganisms have been ranked as being of special concern because of their virulence power and/or the presence of extremely high levels of antibiotic resistance.

Regarding antibiotic resistance, the number of pathogens (belonging or not to the ESKAPE group) harboring ESBLs has dramatically increased worldwide, and these pathogens are associated with high mortality rates in critically ill patients (33). In addition, a continuous spread of carbapenemase-producing *Enterobacteriaceae* isolates has become a major threat to public health (44).

Enterococcus faecium

Enterococcus faecium is a Gram-positive facultative anaerobe that inhabits the gut of humans and animals, and it is an important pathogen in hospitalized patients. *Enterococcus spp.* represents the third most prevalent nosocomial pathogen worldwide, leading related HAI with the emergence of multidrug-resistant vancomycin-resistant isolates (VRE) (45). It is important to highlight the high plasticity of its genome to adapt to different environments, including antibiotic pressure, as demonstrated by the clonal complex CC17, which is highly resistant to ampicillin, fluoroquinolones and vancomycin that are the antibiotics most frequently used in ICUs worldwide (46).

Enterococcus faecium is intrinsically resistant to cephalosporins by the production of a penicillin-binding protein (PBP) with low affinity and different degrees of resistance (MICs from 4 to 256 mg/L) to several aminoglycosides due to limited drug uptake, unlike the MIC values >2,000 mg/L that are described in clinical isolates (47). Furthermore, the presence of the chromosomal encoded AAC(6´)-Ii, responsible for enzymatic inactivation and EfmM ribosomal methylation contributes to the presence of moderate levels of intrinsic resistance to tobramycin (48).

The acquired resistance to ampicillin, penicillin, aminoglycosides, and glycopeptides has been reported in an increasing number of isolates, making it difficult to choose a potentially successful treatment in an ICU. The acquired resistance to glycopeptides is related to different operons (*vanA*, *B*, *D*, *M* and *N*) which result in different levels of resistance (48). Regarding fluoroquinolones, point mutations in the *gyrA* and *parC* genes encoding subunits A of DNA gyrase and topoisomerase IV, respectively lead to high-levels of resistance. Moreover, some findings strongly suggest the presence of a NorA-like efflux pump in *Enterococcus faecium*, which may be involved in resistance to hydrophilic fluoroquinolones (49). Additionally, *Enterococcus faecium* harbors point mutations in the *23S rRNA* gene that confer variable resistance to linezolid and macrolides based on the number of mutated copies of the gene. Nowadays,

daptomycin and tigecycline are alternative treatments, which still retain good levels of activity (25,50).

Staphylococcus aureus

Staphylococcus aureus is a Gram-positive coccus that is a natural inhabitant of the skin microbiota. Nevertheless, *Staphylococcus aureus*, causing frequent chronic infections due to its biofilm formation capacity, which allows *Staphylococcus aureus* to colonize medical devices used in ICUs (51). Resistance to methicillin defines the most classical pathogenic *Staphylococcus aureus*, methicillin-resistant *Staphylococcus aureus* (MRSA), which emerged in the 1960s. The relevance of this microorganism has increased over time with a high prevalence of MRSA being described in some areas. Vancomycin and teicoplanin are used as first-line antibiotics for the treatment of MRSA infections, although vancomycin-intermediate and vancomycin-resistant *Staphylococcus aureus* (VISA and VRSA) containing the *vanA* resistance determinant have been reported. Moreover, VRSA isolates harbor both the *mecA* and *vanA* genes, described as having the same mechanisms as MRSA and VRE, respectively (52,53).

In addition to tigecycline (50), recently, a series of new cephalosporins including ceftaroline or combinations of cephalosporins with β-lactam inhibitors such as ceftazidime-avibactam or ceftozolane-tazobactam which present enhanced activity against *Staphylococcus aureus* have been added to the armamentarium proposed to fight *Staphylococcus aureus* infections (54). Indeed, the use of ceftaroline has been studied both alone and in synergic combination with other antimicrobial agents such as daptomycin (55,56). Nonetheless, despite the short time since the introduction of ceftaroline, the presence of resistance to this antimicrobial has already been described in *Staphylococcus aureus* clinical isolates (57).

Klebsiella pneumoniae

Klebsiella pneumoniae, together with other Enterobacterias such as *Escherichia coli* and *Enterobacter spp.*, have developed resistance against third-generation cephalosporins, due to the explosive spread of ESBLs from which more than 700 variants have been identified, and the number continues to increase worldwide. Additionally, ESBL can often be mobilized through genetic elements, such as plasmids, contributing to fast and easy dissemination between different species. Finally, encoded within the same genetic structure

other antibiotic resistance genes (e.g., aminoglycoside modifying enzymes) are frequently cotransmitted, strongly contributing to the development of multiresistance (58). Thus, *Klebsiella pneumoniae* is considered an alarming health threat, often showing resistance to penicillins, cephalosporins and other antimicrobial agents such as aminoglycosides or carbapenems (58). Resistance to these latter antimicrobial agents is of special concern as this represents the loss of one of the most potent antibacterial agents' families largely used in ICUs. Carbapenems were the first option to treat ESBL-producing bacteria, but the emergence of carbapenem-resistant isolates has now limited this treatment, and polymyxins remain as the last option. Unfortunately, everyday new *Klebsiella pneumoniae* isolates which are resistant to all the antibacterial agents tested are reported, also including polymyxin B and tigecycline (59).

Regarding the molecular mechanisms of antibiotic resistance, the class D extended-spectrum oxacillinase, bla_{OXA-48}, and especially different variants of the class A β-lactamases, KPC, are the most commonly described (60). Aminoglycoside resistance is generally mediated by aminoglycoside modifying enzymes, often encoded within the integron environment, together with other unrelated antibiotic resistance determinants (61). Regarding polymyxin, the most frequently described mechanisms of resistance in *Klebsiella pneumoniae* are related to alterations in the structure of the lipolysaccharide (LPS), with mutations in the *phoP*/*phoQ* and *pmrA*/*pmrB* genes. Moreover, disruption of the *mgrB* gene seems to be the most important mechanism in polymyxin resistance in this bacterium (62).

Acinetobacter baumannii

Acinetobacter baumannii is an opportunistic nocosomial pathogen that presents high resistance to extreme conditions of dryness, changes in pH and high temperature and is able to survive in adverse conditions for a long period of time. This ability makes *Acinetobacter baumannii* the most persistent bacterium in ICUs, being difficult to eradicate, and typically related to ventilator-associated pneumonia and bacteremia (63).

The levels of antimicrobial resistance in *Acinetobacter baumannii* isolates are worrisome. An increasing number of carbapenem-resistant *Acinetobacter baumannii* outbreaks have been reported. These isolates are almost always multidrug resistant, often being extremely drug resistant and even pan drug resistant. The latter show resistance to

all the antimicrobials available.

In *Acinetobacter baumannii* carbapenem resistance is frequently mediated by class D β-lactamases, mainly belonging to the OXA-type, as well as by different class B metallo-beta-lactamases (MBLs) and bla_{KPC}. Thus, *Acinetobacter baumannii* possesses an intrinsic chromosomally encoded oxacillinase bla_{OXA-51}. In addition, a series of acquired carbapenem oxacillinases may also be present, including OXA-23, OXA-24, OXA-58, OXA-143 or OXA-235, among others (64).

In contrast to *Enterobacteriaceae*, the presence of a single mutation in the *gyrA* gene has been related to the development of high fluoroquinolone resistance levels in both *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. This finding has been associated with low membrane permeability as well as with active fluoroquinolone efflux (65). In addition, the upregulation of the AdeABC and AdeIJK efflux-pump systems has been associated with the development of tigecycline resistance (66). Finally, colistin resistance has been linked with the presence of mutations in *pmrAB*, especially with alterations in lipid A of the LPS related to an altered *pmrB* gene (67).

Pseudomonas aeruginosa

Pseudomonas aeruginosa is an opportunistic Gram-negative pathogen and biofilm former able to survive in the mucous of the lungs of patients with cystic fibrosis. It is intrinsically resistant to many antimicrobials and easily acquires additional resistance genes via horizontal gene transfer of mobile genetic elements, especially in ICUs. From the perspective of pediatric patients, emerging meropenem resistance has been described in *Pseudomonas aeruginosa* in nosocomial UTIs (68). *Pseudomonas aeruginosa* may present intrinsic chromosomal overexpression of AmpC, which can contribute to cephalosporin and carbapenem resistance. Furthermore, alterations in the OprD porin leading to non-functional proteins prevent carbapenem uptake and result in carbapenem-resistant isolates (69). Moreover, a high variety of serin carbapenemases and MBLs have been described, including IMP, VIM, KPC, PER and SPM, among others. These carbapenemases are frequently described in relations to plasmid dissemination (70). Furthermore, a series of other class D β-lactamases able to hydrolyze extended-spectrum cephalosporins have also been found in *Pseudomonas aeruginosa*. Additionally, *Pseudomonas aeruginosa* naturally produces the class D β-lactamase OXA-50, which, despite playing a null role in the

development of carbapenem resistance in natural conditions (71), may acquire certain relevance in specific genetic backgrounds (72).

Similar to what occurs with *Acinetobacter baumannii*, polymyxin resistance in *Pseudomonas aeruginosa* is mainly related to modifications in the biosynthesis process of lipid A from LPS that reduces the negative charge of the outer membrane related to the PmrAB, PhoPQ, ParRS, CprRS, and ColRS system (73). Resistance to other antipseudomonal agents, such as aminoglycosides, is also frequent and is mainly related to the presence of aminoglycoside modifying enzymes.

Enterobacter spp.

Enterobacter is an opportunistic Gram-negative pathogen that is also frequent as a cause of HAIs in hospital settings, with a special incidence in ICUs, colonizing both patients and the environment, and leading to mortality rates >20% (74). Furthermore, it has been shown that the eclosion of multidrug-resistant *Enterobacter spp.* isolates has had a negative impact on the clinical outcome of infected patients, increasing mortality rates (75). Nevertheless, it seems that this impact is not directly related to multidrug resistance but rather to the presence of more virulent *Enterobacter spp.* "high risk" clones (75). Currently, the presence of *Enterobacter spp.* exhibiting resistance to all the scheduled antibiotics except colistin and/or tigecycline is not rare (76). Consequently, a pleiad of antibiotic resistance mechanisms are present within the genome (chromosome or plasmids) of these microorganisms (77).

Among the most relevant mechanisms of antibiotic resistance described in *Enterobacter spp.*, a large variety of plasmid-mediated AmpC (pAmpC) including bla_{DHA} and bla_{CMY}, ESBLs including bla_{SHV}, bla_{TEM} or bla_{CTX-M} and carbapenemases, including mainly bla_{KPC} and bla_{OXA} and MBLs underlie the resistance to cephalosporins and carbapenems (75,77,78). Furthermore, *Enterobacter spp.* are intrinsically resistant to aminopenicillins, cefazolin, and cefoxitin due to the production of constitutive chromosomal AmpC (79,80). These microorganisms also possess a chromosomally encoded *qnr* gene (81), which probably confers a favorable genetic background for enhanced development of further quinolone-targeted mutations and subsequent resistance to fluoroquinolones.

Regarding last resort antibiotics, the presence of *Enterobacter spp.* resistant to tigecycline or colistin due to impaired uptake and enhanced pump out has been

reported (78). Meanwhile, hampered intake as well as mutations of the *pmrAB* and *phoPQ* genes have been reported to lead to colistin hetero resistance (82,83). Furthermore, the presence of transferable mechanisms of colistin resistance such as *mcr1* has also been reported in *Enterobacter spp*. from hospital environments (84).

Escherichia coli

Although is a commensal member of gut microbiota, *Escherichia coli* is the most common pathogen causing bacteremia, and urinary and catheter-related infections, ranking as an important cause of morbidity and mortality worldwide, especially in ICUs. This double role has a direct translation in ICUs, in which *Escherichia coli* strains may be a direct disease agent as well as a Trojan horse which introduces a series of antibiotic-resistant determinants into the ICU (61). These determinants may be horizontally disseminated to other pathogenic or non-pathogenic microorganisms. This finding highlights the fact that ICUs are not isolated islands and that community antibiotic use also has an impact on the levels and mechanisms of antibiotic resistance detected in ICUs (39). It is of note that from a pediatric point of view, higher rates of ertapenem resistance in ESBL positive *Escherichia coli* isolates (and *Klebsiella spp*.) in pediatric nosocomial UTIs are important signs for indicating the development of superbug infections (68).

Among the most relevant mechanisms of resistance, *Escherichia coli* strains harbor different ESBLs, which are frequently encoded within the same genetic structure of transferable mechanisms of quinolone resistance (TMQR) or aminoglycosides, among others. This finding leads to the so-called antibiotic resistance co-selection. Regarding quinolones, as commented above regarding the chromosome-encoded *qnr* gene of *Enterobacter spp*., the presence of TMQR favors the selection of highly quinolone-resistant mutants related to mutations in the *gyrA* and/or *parC* genes (85).

Regarding mechanisms of resistance to other antibiotics used in ICUs, the presence of carbapenemases such as *bla*$_{NDM}$ or *bla*$_{CTX-M}$, among others, is increasingly being reported (86,87), while a variety of aminoglycoside modifying enzymes is frequently detected, mostly within integrons (61). Furthermore, the presence of a polymyxin resistance gene in mobile determinants, such as *mcr* has recently been described (86,88), being by far one of the most relevant issues related to the emerging antibiotic resistance.

Conclusions

Increasing levels of antimicrobial resistance are a matter of concern in all environments, but in ICU settings this phenomenon is especially worrisome because of the fragile situation of the patients admitted to these units. In these settings, the establishment of a multidrug resistant hospital-adapted pathogen may lead to an increasing number of fatal outcomes.

The continuous description of new bacterial adaptations to current antimicrobial agents, together with the accumulation of antibiotic resistant determinants within the same genetic structures and the exchange of this genetic material between closely and far phylogenetically related microorganisms warn of the possible return of untreatable outbreak infections. In fact, the presence of pandrug-resistant microorganisms inside and outside ICUs has been reported.

No definitive measure to eradicate antibiotic-resistant microorganisms from ICUs has been designed and only approaches to limit their impact have been developed. In this scenario, extreme hygiene measures in ICUs, avoidance of the presence of unnecessary personnel and clinical devices and the definitive implementation of strict control of antibiotic access in all human and veterinary activities are unnegotiable in order to adequately control the spread of the current epidemic of antibiotic resistance.

While vaccine development against some pathogens may limit their presence in ICUs, it is unaffordable to develop and implement vaccination approaches against all the current bacterial pathogens. In this scenario, the development of new antibacterial agents addressing unexplored bacterial targets or the development of novel treatment approaches, such as the use of phagotherapy, may be the road to future safe ICUs.

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Footnote

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