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Bugs, hosts and ICU environment: Countering pan-resistance in nosocomial microbiota and treating bacterial infections in the critical care setting

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ABSTRACT

ICUs are areas where resistance problems are the largest, and they constitutes a major problem for the intensivist's clinical practice. Main resistance phenotypes among nosocomial microbiota are: i) vancomycin-resistance/heteroresistance and tolerance in grampositives (MRSA, enterococci) and ii) efflux pumps/enzymatic resistance mechanisms (ESBLs, AmpC, metallobetalactamases) in gramnegatives. These phenotypes are found at different rates in pathogens causing respiratory (nosocomial pneumonia/ventilator-associated pneumonia), bloodstream (primary bacteremia/catheter-associated bacteremia), urinary, intraabdominal and surgical wound infections and endocarditis in the ICU. New antibiotics are available to overcome non-susceptibility in grampositives; however, accumulation of resistance traits in gramnegatives has lead to multidrug resistance, a worrisome problem nowadays. This article reviews by microorganism/infection risk factors for multidrug resistance, suggesting adequate empirical treatments. Drugs, patient and environmental factors all play a role in the decision to prescribe/recommend antibiotic regimens in the specific ICU patient, implying that intensivists should be familiar with available drugs, environmental epidemiology and patient factors.

Key words: MRSA; vancomycin-resistant enterococci; ESBL; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; critical care

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Gérmenes, huéspedes y el entorno de la UCI: Contrarrestando la panresistencia en la microbiota nosocomial para tratar las infecciones bacterianas en cuidados críticos

RESUMEN

Las UCI son las áreas con mayor problema de resistencias, y constituye uno de los principales problemas de los intensivistas en su práctica clínica. Los principales fenotipos de resistencia en la microbiota nosocomial son: i) la resistencia/heteroresistencia y la tolerancia a la vancomicina en grampositivos (SARM, enterococo) y ii) las bombas de eflujo/mecanismos enzimáticos de resistencia (BLEEs, AmpC, metalobetalactamasas) en gramnegativos. Estos fenotipos pueden encontrarse, con distinta frecuencia, en patógenos causantes de infecciones respiratorias (neumonía nosocomial/neumonía asociada a ventilación mecánica), del torrente sanguíneo (bacteriemia primaria/bacteriemia asociada a cateter), urinarias, intraabdominales, de herida quirúrgica y endocarditis en la UCI. Hay nuevos antibióticos disponibles para contrarrestar la no-sensibilidad en grampositivos; sin embargo, la acumulación de factores de resistencia en gramnegativos lleva a la multirresistencia/panresistencia, un problema en nuestros días. Este artículo revisa por microorganismo/infección los factores de riesgo de resistencia/multirresistencia, sugiriendo tratamientos empíricos adecuados. Fármacos, pacientes y factores ambientales tienen todos un papel básico en la decisión de prescribir/recomendar regímenes antibióticos en el paciente específico de la UCI, implicando que los intensivistas deben estar familiarizados con los fármacos disponibles, la epidemiología local y las características del paciente crítico.

Palabras clave: SARM; enterococo resistente a vancomicina; BLEE; *Pseudomonas aeruginosa*; *Acinetobacter baumannii*; cuidados críticos

THE NOSOCOMIAL MICROBIOME AND RESISTOME

Evolution of relationships between human and bacteria are conditioned by environmental changes. Among anthropogenic factors changing the environment and thus, shaping future interactions between human and bacteria[1,2], chemical pollution (including antibiotics and antimicrobial strategies) altering microbial biodiversity, new medical technologies (opening the way for opportunistic infections), the increasing number of highly susceptible hosts and control of bacterial access to host are important factors for nosocomial infections, and theoretically, counterbalance colonisation/multidrug resistance in nosocomial microbiota.

The "nosocomial human population", that includes patients and health care personnel, is closely linked to the "nosocomial microbiome" (microbiota from health care personnel and from non-infected and infected patients), with its specific "resistome" (antibiotic resistance genes and genetic elements that participate in resistance gene transfer). The horizontal gene transfer within species and between different species of gram-negative and gram-positive bacteria[3], facilitated when bacteria are exposed to antibiotic stress[1,4,5], has driven to multidrug resistance.

Resistance implies the need for new antibiotics that, once introduced, if their mechanism of action is similar to previous compounds may select pre-existing resistances or induce new resistances in the nosocomial resistome that could be further selected, thus implying the need for new antibiotics and closing the circle[6]. Antimicrobial pressure as driving engine for resistance and multidrug resistance is evident in the nosocomial environment, with a well defined relationship between antibiotic use and emergence of multidrug resistant strains[7-9].

In the presence of antibiotic stress, antimicrobial resistance can be considered a colonisation factor[1]. Accumulation of "genotypic colonisation factors" (phenotypic resistance traits) drives to multidrug resistance, hallmark of nosocomial microbiota since the phenomena of selection of co-resistance and co-selection of resistance are more frequent in hospitals than in the community. If resistance favours "colonisation" of elements of the nosocomial microbiota, strategies aimed to reducing resistance will result not only in a decrease in the resistance prevalence but also in a decrease in colonisation and a subsequent decrease in nosocomial infections.

Hospital-acquired infections affect a quarter of critically ill patients, and can double the risk of a patient dying[10,11], requiring rapid treatment to reduce morbidity and mortality[12]. Nosocomial infections acquired in the intensive care unit (ICU) represent an area in which much improvement is still achievable[13]. However it should be taken into account that infection is often the cause of ICU admission[14,15], influencing the microbiological environment of the unit[16]. The drugs, patient and environmental factors all play a role in the decision to prescribe or recommend (and daily review) antibiotic dosing regimens in a specific patient[12], this implying that personnel involved should be familiar with available drugs, environmental (bacterial epidemiology and resistance traits) and patient factors.

The concrete battlefields in the ICU

An approach to the existing resistome can be done through the choice of indicator microorganisms based on their clinical relevance and their potential for acquisition of genetic determinants of resistance. Nowadays, the main resistance phenotypes among multiresistant nosocomial microbiota are: i) vancomycin-resistance and tolerance in nosocomial gram-positives (MRSA and enterococci) and ii) efflux pumps and enzymatic resistance mechanisms (ESBLs, AmpC and metallobetalactamases) in nosocomial gram-negative bacteria. Antibiotics/antibiotic regimens for the treatment of nosocomial infections should counter these sometimes emerging, always diffusible and clinically worrisome resistance traits.

THE METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) CASE

Staphylococcal infections became treatable with the introduction of penicillin but, soon after, production of β-lactamase by staphylococci became a reality. Penicillinase-resistant isoxazolyl penicillins were then introduced to counter resistance mediated by β-lactamases, with the subsequent emergence of methicillin resistance. Nowadays, MRSA is worldwide spread in hospitals, with prevalence reaching rates of 25-50% in much of Americas, Australia and Southern Europe[17]. The evolution of the global rate of MRSA among *S. aureus* in Spanish ICUs from 1994 to 2008 (study ENVIN-UCI including up to 100 ICUs) shows similar rates (≈25%) in the first and last years with oscillations ranging from 13% in 1997 to 42.3% in 2006[18]. In addition to intra-ICU transmission dynamics of MRSA (influenced, among others, by colonisation of health care workers in the ICU[19]), it should be taken into account MRSA imported cases in the ICU as predictor of occurrence of nosocomial MRSA infections[20], with community-acquired MRSA genotypes as emerging cause of colonisation among patients admitted in adult ICUs in the USA[21].

The dramatic increase in MRSA nosocomial infections led to a substantial increase in the use of vancomycin, and this could be related to the appearance of different vancomycin non-susceptible phenotypes both in enterococci and staphylococci. The risk of emergence of MRSA non-susceptible to vancomycin is much higher in countries with high prevalence of both MRSA and vancomycin-resistant enterococci[22]. Published studies suggest a link between antibiotic usage at individual and institutional levels and resistance, showing an increase in the risk of acquiring MRSA when using not only glycopeptides[23] but also quinolones[23,24] and cephalosporins[24,25].

The associated problem of vancomycin non-susceptibility and vancomycin tolerance

The first vancomycin non-susceptible strains were designated as vancomycin-intermediate *S. aureus* with vancomycin MICs of 8-16 mg/L[26]. Among vancomycin-intermediate strains, 90% of strains are heterogeneous vancomycin-intermediate (heteroresistant; h-VISA) characterized by the presence of a

selectable resistant subpopulation in an otherwise fully susceptible population, and only 10% are homogenously vancomycin-intermediate (homoresistant; VISA)[27,28]. The prevalence of h-VISA among MRSA is variable worldwide ranging from ≈10 to 50%[29-32]. MRSA strains resistant to vancomycin have been described but, fortunately, its diffusion is unappreciable nowadays[33]. Intermediate resistance to vancomycin can also be found in coagulase-negative staphylococci at non negligible rates (≈10%)[34].

In addition, there are MRSA isolates that are susceptible to vancomycin but tolerant to its killing effect. Tolerance is defined as "bacterial capability of survival without growth in the presence of a current lethal concentration"[35], and is expressed as an MBC/MIC quotient of ≥16 or ≥32[36]. Nevertheless, a recent study has shown that even vancomycin-susceptible strains with MBC/MIC ratios of 8, when exposed to simulated vancomycin concentrations in serum, exhibit a pharmacodynamic behaviour similar to that of strains with MBC/MIC ≥16, with no bactericidal activity by vancomycin despite susceptibility[37]. Tolerance to vancomycin is present in 100% VISA strains, 75% h-VISA and 15% vancomycin-susceptible MRSA[36] and this phenomenon is extensive to other glycopeptides as teicoplanin, with teicoplanin tolerance reported in 18.8% of MRSA strains[38]. In addition, tolerance to glycopeptides has also been described in ≈25% of coagulase negative staphylococci and ≥40% of group viridans (*Streptococcus bovis*, *Streptococcus sanguis*, *Streptococcus gordonii*, *Streptococcus mutans* and *Streptococcus oralis*) isolates[34], both bacterial groups being important etiological agents in endocarditis.

Clinical impact of non-susceptibility, resistance and/or tolerance

Bactericidal activity is important in infections caused by methicillin-susceptible *S. aureus*[39]. A classical study in our country showed a significantly higher mortality in methicillin-susceptible *S. aureus* bacteremia in patients treated with vancomycin compared with cloxacillin, in part attributable to the slow vancomycin killing[40]. This was corroborated in an in vitro study showing that vancomycin was not bactericidal within the dosing interval in contrast to daptomycin, regardless methicillin susceptibility/resistance of the study strains[41].

Some high-inoculum staphylococcal infections as bacteremia, persistent bacteremia, endocarditis and osteomyelitis have been associated with heteroresistance[33,42,43]. Vancomycin heteroresistance has been linked to strains susceptible to vancomycin but with high MIC values within the susceptibility category[44,45]. In turn, the relationship of MICs to clinical failure with vancomycin is striking[31]. In a published study, high vancomycin MICs, defined as 1.5-2.0 mg/L, was an independent predictor of poor response to vancomycin therapy for MRSA infection, even when vancomycin trough levels >15 mg/L were achieved[46]. Importantly, vancomycin trough levels >15 mg/L appears to be associated with a 3-fold increased risk of nephrotoxicity[47].

Considering the current situation, it has been suggested that strains with vancomycin MIC of 1-2 mg/L should be considered h-VISA or VISA[48] since even the new Clinical and Laboratory Standards Institute (CLSI) susceptibility breakpoint for vancomycin (≤2 mg/L) may fail to precisely differentiate potential responders to vancomycin therapy[36,49], suggesting that, according to clinical data, the breakpoint value should be even lowered to 1 or 0.5 mg/L[48].

The spectrum of clinical disease caused by MRSA, h-VISA, VISA and tolerant isolates is similar to that caused by non-tolerant methicillin-susceptible *S. aureus*. Since antimicrobial treatment is empirically initiated, there is evidence to show that less than a quarter of patients with MRSA infections receive correct therapy within 48h of hospital admission, and only ≈40% receive appropriate agents after 48h[50]. Clinical implications of heteroresistance and tolerance evidenced as poor clinical outcome, persistence of bacteremia and increased length of stay[24,51-55], together with the fact that these phenomena are not routinely tested by microbiologists and reported to treating physicians[34], stress the importance of therapeutic strategies to overcome them.

THE VANCOMYCIN-RESISTANT ENTEROCOCCUS (VRE) CASE

Enterococci, historically regarded as a second-rate pathogen and with low virulence, have become one of the most challenging nosocomial problems. Nowadays, *Enterococcus faecium* is almost as common as *Enterococcus faecalis* as a cause of nosocomial infection[56]. All enterococci show tolerance to vancomycin[57]. In addition, acquisition of resistance to ampicillin, aminoglycosides (high level) and glycopeptides in *E. faecium* is a cause of concern[22], making *E. faecium* infections difficult to treat. In USA vancomycin resistance increased in *E. faecium* isolates from 0% in mid 1980s to 80% in 2007[58]. In Europe the vancomycin resistance prevalence is variable, ranging from <1% to >40%[22,59]. In Spain rates of around 14.3% have been reported in *E. faecium*[60]. At hospital level, the increase in vancomycin use to treat MRSA infections seems to be the origin of VRE. In addition, the intensive use of oral vancomycin for *Clostridium difficile* infections in hospitals is also likely to select and increase faecal carriage of VRE[3]. In this sense, the description of multidrug resistant, hospital-adapted *E. faecium* clonal complexes without community reservoir can be explained by cross-transmission, selection and diffusion by selective antibiotic pressure[61]. Factors associated with VRE colonisation in critically ill patients include prolonged ICU stay (each day in the ICU increases 1.03 times the risk of acquisition), previous antibiotic use and carbapenem use[62-64]. Risk factors for development of VRE infections include prolonged hospitalisation, surgical or intensive care units, intravascular or bladder catheter devices, proportion of colonised patients and exposure to antibiotics[65,66]. Among antibiotics, in addition to vancomycin, certain compounds as ticarcillin/clavulanate and third generation cephalosporins have demonstrated to cause selection[67,68]. Although initially hospital-associated clones were different than those community-associated, these later

have become important nosocomial pathogens[58], with colonisation prior to ICU admission being associated with previous hospitalisation and, again, antibiotic exposure[69].

In enterococci full resistance to daptomycin, although has been reported[70], is rare, as for linezolid[58]. The increase in linezolid use has been related to an increase (and to outbreaks) of VRE resistant to linezolid[71-73], also in patients not previously exposed to the drug[74].

Clinical outcomes are worse and mortality higher in patients with VRE infections when compared to those infected by susceptible strains[66]. The classical tolerance to the killing capability of penicillins and glycopeptides in enterococci has clinical implications, as evidenced in enterococcal endocarditis where, due to the historical high recurrence rates with penicillin or glycopeptide monotherapy, combined therapy (including an aminoglycoside) is the rule[75]. However, nowadays, due to the high aminoglycoside resistance in VRE, recurrences can occur.

STRATEGIES TO OVERCOME NON-SUSCEPTIBILITY PHENOTYPES IN MRSA AND VRE

Compromise of the bactericidal activity, among other factors, by vancomycin heteroresistance/tolerance (MRSA) or tolerance/resistance (VRE) may have clinical implications. Conceptually, treatments achieving bactericidal activity are preferred than those only presenting bacteriostatic activity, although this has not been clearly demonstrated in clinical trials. There are clinical indications where it is considered that bactericidal activity is absolutely necessary, as bacteremia, endocarditis, meningitis and infections in immunocompromised patients[39].

Two strategies can be considered to overcome deterioration of bactericidal activity by non-susceptible phenotypes: i) combined therapy obtaining synergism and ii) bactericidal antibiotics for initial treatment.

The addition of a new antimicrobial is outlined when facing a poor response with vancomycin monotherapy, thus suggesting tolerance of the infecting strain[55], and has been successful used in the treatment of refractory bacteremia by tolerant isolates[76]. The election of drugs to be included in the combination is important since there have been described antagonic interactions between linezolid and vancomycin or between linezolid and gentamicin[77,78], on one side, and the commented high aminoglycoside resistance in VRE on the other. No antagonistic interactions have been shown between daptomycin and gentamicin, linezolid or vancomycin[79].

Regarding initiation of antibiotic therapy with bactericidal drugs, among compounds with potential activity against gram-positives, it should be taken into account that linezolid and tigecycline are bacteriostatic against *S. aureus*, and that quinupristin/dalfopristin, although bactericidal against *S. aureus*, is bacteriostatic against *E. faecium* and non active against *E. faecalis*[27]. Bactericidal compounds to be used should present activity against gram-positive isolates and lack of tolerance or heteroresistance, in contrast to glycopeptides, as the lipopeptide daptomycin that represents an adequate option for initial treatment of nosocomial gram-positive infections as staphylococcal bacteremia, endocarditis and skin and soft-tissue infections, but not of pneumonia due to the inhibition of its antibacterial activity by the pulmonary surfactant.

THE EXTENDED SPECTRUM β-LACTAMASE (ESBL), AMPC AND CARBAPENEMASES CASE

Pan-resistance is an increasing problem among nosocomial gram-negatives mainly due to antibiotic inactivating enzymes, sometimes in combination with efflux pumps and/or porine deficits. *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* are specifically addressed as the most problematic and often extensively or pan-drug resistant pathogens[80]. In Spain, the proportion of *A. baumannii* isolates showing resistance to carbapenems, ceftazidime, aminoglycosides and quinolones is around 50% (for the first three) and 87% for ciprofloxacin, and in *P. aeruginosa* isolates proportions are ≈20% (carbapenems), ≈15% (ceftazidime) and ≈25% (aminoglycosides and quinolones)[80]. In *K. pneumoniae*, resistance to third generation cephalosporins and aminoglycosides is ≈10% and ≈18% for quinolones[80].

Different types of β-lactamases are increasingly appearing and diffusing as response to antibiotic pressure at the nosocomial level. In general, β-lactamases diffusing among human microbiota may be classified into three groups: 1) Extended-spectrum β-lactamases (ESBL), 2) AmpC and 3) Carbapenemases.

1) ESBL

After the introduction in the 80's of extended-spectrum third-generation cephalosporins, mutations in both bla_{TEM} and bla_{SHV} genes were reported, mainly in *Klebsiella* spp. In the last decade, there has been a rise in the prevalence of CTX-M β-lactamases that, unlike TEM and SHV ESBLs, did not remain confined to *Klebsiella* and have proliferated in *Escherichia coli*[81]. In Spain the prevalence of ESBL-producing *E. coli* has 8-fold increased from 2000 to 2006[82]; the SMART study reported a frequency of ESBL-producing isolates of ≈8.5% for *E. coli* and for *Klebsiella* spp.[83]. Urine, followed by blood, and internal medicine, general surgery and ICUs were the most common sites and wards of isolation, respectively, in another study[84].

The huge amount of molecular variants widely diffused around the world is creating problems in the treatment of nosocomial infections since these enzymes are capable to confer resistance to penicillins, first-, second- and third- generation cephalosporins and to aztreonam (but not to cephamycins and carbapenems), but can be inhibited by β-lactamase inhibitors[85]. However, non-susceptibility rates (according to EUCAST breakpoints) to piperacillin/tazobactam in CTX-M-producing *E. coli* and *K. pneumoniae* were 27.4% and 38.1%, respectively, with high resistance rates to cefepime (≈70% and ≈80%, respectively)[86]. In addition, in ESBL-producing strains co-resistance to aminoglycosides and quinolones is present[85]. Due to this,

ESBL-producing strains have been clearly associated with poor outcome. In this sense, empirical therapy with cephalosporins or fluoroquinolones was associated with a higher mortality compared with patients treated with a β-lactam/β-lactamase inhibitor or with carbapenem-based regimens in a Spanish series of patients with bacteremia produced by ESBL-producing *E. coli*[87].

Selection and diffusion of ESBLs has been associated with antibiotic pressure derived from the use of third-generation cephalosporins (with special importance for ceftazidime), aminoglycosides and quinolones, but not to β-lactams/β-lactam inhibitors or carbapenems[85]. In addition to previous antibiotic treatments, other risk factors that have been described for infection by ESBL-producing isolates in ICU patients are previous hospitalisation, advanced age, diabetes and use of catheters[84].

Carbapenems are probably the best options for treating infections caused by ESBL-producing strains[84,87], but the risk of the emergence of carbapenem resistance should always be considered (see below).

2) AmpC

Isolates of *Enterobacter cloacae*, *Enterobacter aerogenes*, *Serratia marcescens*, *Citrobacter freundii*, *Providencia rettgeri* and *Morganella morganii* (known as the ESCPM group) have the potential to produce AmpC inducible chromosomal β-lactamases upon exposure to inducing agents: aminopenicillins, first-generation cephalosporins, cephamycins and carbapenems as strong inducers, and second- or third-generation cephalosporins, acylureidopenicillins or monobactams as weakly inducers[88,89]. When the inducer is removed, AmpC production returns to hardly detectable basal levels; thus, when isolated from patients, bacteria are found to be susceptible to third-generation cephalosporins. However, AmpC production should be suspected in all isolates belonging to these species. When inducer drugs are clinically used, selection of derepressed mutants (constitutively producing β-lactamase) occurs, with contingent clinical failure[89,90]. An association between the use of third-generation cephalosporins and the emergence of resistance has been established among organisms with inducible chromosomally encoded AmpC β-lactamases[90]. Derepressed overproduction has been described in 20% infections by *Citrobacter* spp. or *Enterobacter* spp. during third-generation cephalosporin treatment[3].

AmpC genes have been mobilized to plasmids and spread worldwide, with increasing numbers in the diversity of this type of enzymes[3]. Infections caused by plasmid AmpC-producing isolates significantly increase treatment failure probably due to inadequate initial treatment therapy[91]. The CLSI provides susceptibility breakpoints for third-generation cephalosporins and AmpC producers but advice that resistance can emerge, and many infectious diseases specialists advocate that these compounds should not be used for significant infections caused by AmpC-producing enterobacteria[92,93]. In addition, ESBLs has been increasingly described in AmpC producers, which further complicate decisions related to the optimum antimicrobial therapy[93].

AmpC- and ESBL- producing isolates exhibit high rates of resistance to penicillins (including piperacillin/tazobactam) and cephalosporins (including cefepime) according to EUCAST breakpoints[86]. Treatment with carbapenems represents a good option but, again, concerns on the potential emergence of carbapenem resistance arise.

3) Carbapenemases

Most carbapenemase-producers have multiple resistance mechanisms to β-lactams and to aminoglycosides[94].

Resistance to carbapenems can arise by:

a) Permeability alterations (efflux pumps and/or porine deficit) plus AmpC (class C β-lactamases) or ESBL (class A) enzymes,

b) Acquisition of non- metallo-carbapenemases mainly of the KPC or OXA (class D β-lactamases) families, and/or

c) Acquisition of metallo-β-lactamases (MBLs; class B β-lactamases), mainly of the IMP- and VIM- families.

The heavily use of carbapenems after dissemination of multidrug resistant *Enterobacteriaceae* (due to ESBL and AmpC β-lactamases) rises the fears of the relationship between the use of these antibiotics and the selection and diffusion of carbapenemase-producing strains. Although nowadays the prevalence of carbapenemases is relatively low, they are sources of considerable concern due to the enzyme spectrum of activity that encompasses almost all known β-lactams, from penicillins to carbapenems, and because they are not susceptible to class A β-lactamase inhibitors and currently there are not clinically available inhibitors to block MBLs action[95]. The association of carbapenemase production to resistance traits to other antibiotic classes may lead to polymyxins and tigecycline as last active agents, neither of them ideal. Resistance mediated by carbapenemases affects primarily *A. baumannii*, *P. aeruginosa* and to lesser extent, *K. pneumoniae,* although its emergence has also been described in *B. fragilis*[96].

P. aeruginosa

Pan-resistance in *P. aeruginosa* results from the convergence of multiple resistance mechanisms[97]: low outer membrane permeability, AmpC β-lactamases, efflux pumps and less often, production of MBLs[97,98]. However, in many European countries, mainly in the Mediterranean area, VIM-type producing *P. aeruginosa* has currently become endemic[99]. In Spain the prevalence of carbapenemase-producing *P. aeruginosa* strains among bacteremic isolates resistant to imipenem has increased 10 times in few years, reaching 4% in 2008[100]. According to the EARSS study, non-susceptibility rates are ≈8% to piperacillin/tazobactam, ≈15% to ceftazidime, ≈25% to aminoglycosides and quinolones, and ≈20% to carbapenems[80]. In the ICU, risk factors for multidrug resistance in *P. aeruginosa* are previous exposure to third-generation cephalosporins, to carbapenems or to acylureidopenicillins[101].

A. baumannii

A. baumannii is more often resistant. *A. baumannii* produces a naturally occurring AmpC β-lactamase, like *P. aeruginosa*, together with a naturally occurring oxacillinase with carbapenemase properties[102]. Additionally, resistance to carbapenems has been linked to the loss of outer membrane porins and upregulated efflux pumps[80]. Resistance to carbapenems remained rare until 2000 despite the widespread of resistance to other compounds[98]. However, carbapenem resistance has increased sharply since then, and is mediated by OXA-type, and less often by IMP- and VIM- types, carbapenemases[80,98]. Several studies have described the OXA-40 gene spread across the Iberian Peninsula[103,104]. In our country, resistance rates are ≈35% to amikacin, ≈40% to ceftazidime, ≈70% to piperacillin/tazobactam, and ≈45% to carbapenems[105]. It is considered that resistance to carbapenems is enough to define an isolate as highly resistant[106]. Risk factors for carbapenem resistance in *A. baumannii* are hospital size, ICUs, length of stay in the ICU, recent surgery, invasive procedures and, mainly, previous exposure to antibiotics (carbapenems and third-generation cephalosporins) and mechanical ventilation[107,108].

Enterobacteriaceae

As previously described, the main multidrug resistance phenotype in enterobacteria is due to hyperproduction of chromosomal AmpC β-lactamases or ESBLs. Undoubtedly, this phenotype is also represented by carbapenem resistance mainly mediated VIM- and IMP- type MBLs. In the *Enterobacteriaceae* family, *K. pneumoniae* is the species with the highest rates of carbapenem resistance. In the multinational SENTRY study (2007-2009), overall carbapenemase resistance in *K. pneumoniae* was 5.3%, while it was 0.3% in *E. coli,* mainly due to KPC β-lactamases in *K. pneumoniae* and OXA-48 in *E. coli*[109]. In Spain, class B carbapenemase-producers (VIM-1 and IMP-22) have been found in specific areas (Madrid, Catalonia, Andalucia, Balearic) with a local prevalence <0.2%[110]. But the situation may be changing since the description of VIM-producers outbreaks[110-114], together with the emergence of the KPC-3[115] and the New Delhi MBL (NDM-I) β-lactamases in *K. pneumoniae* and *E. coli*[116,117], confirming the dissemination of carbapenemase-producing isolates in our country. Nonetheless, according to last EARSS data in 2011, carbapenemase resistance in *K. pneumoniae* in Spain is 0.3%[118]. Risk factors associated with carbapenem resistance in *K. pneumoniae* are previous exposure to antibiotics (carbapenems, cephalosporins, acylureidopenicillins and quinolones), mechanical ventilation, and stay in the ICU[80,119-121]. Carbapenem-resistant *K. pneumoniae* has been independently associated with poor outcome and death[120,122,123].

PHARMACOKINETICS/PHARMACODYNAMICS (PK/PD) IN THE ICU SETTING

The choice of an antibiotic for empirical treatment of serious bacterial infections in the ICU is based predominantly in the identity and susceptibility patterns of bacteria commonly isolated in a particular ICU. Serious infections in critically ill patients require rapid treatment to limit morbidity and mortality. Intravenous treatment should begin within the first hour after diagnosis of severe sepsis[124] as the most important factor affecting outcome. However, this is not always met since as few as 25% of the first doses of antibiotics are administered within 1h of prescription[12]. What is often overlooked is the optimum dose of an antibiotic[12] and, to avoid empiricism, the PK/PD relation should be exploited[80]. However, PK/PD parameters predicting efficacy usually rely on steady-state concentrations, avoiding events occurring when the pathogen is exposed to the initial dose, which are relevant for outcome[125]. Ideally, the first dose should rapidly reach enough concentrations above the MIC to avoid resistance selection, and these concentrations should be maintained all over the treatment course. In order to escape resistance, under-dosing should be avoided and the duration of therapy should be limited, starting de-escalation of administered antibiotics as soon as culture results are ready[80]. Considering all these facts and the challenging situation of resistances, the role of clinicians is currently enhanced since they are vital resource in the implementation of strategies against worrisome pathogens.

From the pharmacodynamic perspective, antimicrobials are basically classified according to the type of antibacterial activity (concentration-dependent or time-dependent) and the presence of post-antibiotic effect (time to bacterial regrowth after elimination of the antibiotic from the media)[126]. According to this, three main groups can be defined:

1) Antibiotics with concentration-dependent activity and prolonged post-antibiotic effect. PK/PD parameters related to efficacy are Cmax/MIC and AUC/MIC. Commonly used antibiotics in the ICU included in this group are: aminoglycosides, fluoroquinolones and daptomycin. Target values of PK/PD parameters are: Cmax/MIC of 10-12 for aminoglycosides, and AUC_{0-24h}/MIC >125 for fluoroquinolones in severe infections and ≥666 for daptomycin[126,127].

2) Antibiotics with time-dependent activity and minimal or moderate post-antibiotic effect. The PK/PD parameter related to efficacy is *f*T>MIC (time that free concentrations exceed the MIC, expressed as % of the dosing interval). Commonly used antibiotics in the ICU included in this group are: penicillins, cephalosporins, monobactams, carbapenems and macrolides, with target values of >50% for penicillins, >60-70% for cephalosporins and monobactams, >30-40% for carbapenems and >40% for macrolides[126,127].

3) Antibiotics with concentration-independent action and prolonged antibiotic effect. The PK/PD parameter related to efficacy is the AUC/MIC. Commonly used antibiotics in the ICU included in this group are: vancomycin, linezolid, azalides and tigecycline, with target values of ≥400 for vancomycin, ≥100 for linezolid, ≥25 for azalides and ≥15-20 for tigecycline[126,127].

Antibiotics belonging to the first group can be used at high doses and the prolonged post-antibiotic effect allows wider dos-

ing intervals. In this sense there is good evidence for extended duration of the dosing interval of aminoglycosides in critically ill patients[12] that, in addition, reduces renal toxicity[127]. For antibiotics in the second group, the objective is the consecution of a long bacterial exposure to the antibiotic; for this reason, continuous infusion (when possible) is the best regimen since antibiotic serum concentrations are constantly above the MIC for the duration of treatment. In an in vitro model the intermittent infusion of ceftazidime provided bactericidal activity against susceptible *P. aeruginosa* strains, but not against resistant strains, and continuous infusion optimised t>MIC and resulted in bactericidal activity[128]. Continuous infusion with an initial loading dose (to rapidly obtain bactericidal concentrations) allows adequate concentrations at steady-state, minimising fluctuations of serum concentrations. However, there are scarce clinical studies demonstrating the better efficacy obtained with continuous versus intermittent infusion; with reports using piperacillin/tazobactam[129] or meropenem[130]. In contrast, no significant differences in outcomes and toxicity between bolus and continuous infusion of β-lactams are usually described, with a lack of studies in the ICU[127].

Finally, for antibiotics in the third group the increase in concentrations only slightly increase bacterial eradication, but highly increase a prolonged inhibition of bacterial growth. One of the principal difficulties for vancomycin dosing is predicting future doses from trough level data in the ICU, and therapeutic drug monitoring is needed[12]. Administration of vancomycin by continuous infusion has been advocated to improve clinical outcome, although data from ICU patient are scarce. A published study showed lower mortality in ICU patients with ventilator-associated pneumonia receiving continuous vancomycin infusion[131]. However, the risk of nephrotoxicity associated with continuous-infusion vancomycin requires further investigation[132] since acute kidney injury was frequently observed during continuous vancomycin infusion in a study in critically ill patients[133]. In the case of linezolid, both AUC/MIC and t>MIC (85%) correlate with eradication and clinical cure in ICU patients[134]. However, interstitial linezolid concentrations in patients with sepsis suffer high inter-individual variability, supporting more frequent dosing schemes to avoid subinhibitory concentrations in infected tissues[135]. Continuous infusion has also been suggested for critically ill patients to obtain more stable linezolid levels and adequate AUC/MIC and t>MIC values[136].

Colistin, a polymyxin agent, is in some cases the last option for the treatment of multidrug resistant *A. baumannii* and *P. aeruginosa*. It exhibits a concentration-dependent activity with prolonged post-antibiotic effect at high concentrations[137]. Due to its poor gastrointestinal absorption and the classically reported nephrotoxicity and neurotoxicity of the intravenous formulation, in the ICU setting colistin is usually used as nebulized drug. However, colistin can be the sole agent active against muti-drug resistant gram-negatives in critical care, and it has been suggested that its toxicity may have been overestimated[137]. The lack of PK/PD data results in a difficulty for optimisation of its daily dose aimed to maximise the AUC/MIC ratio, parameter best associated with colistin efficacy[138].

In critically ill patients, in addition to alterations in hepatic or renal functions, variations in the extravascular fluid affect drug disposition. Hydrophilic drugs (β-lactams, aminoglycosides and glycopeptides) and renally excreted moderately lipophilic agents (quinolones) have a considerable risk of presenting daily fluctuations in plasma concentrations that may require dose adjustments[139]. Hydrophilic compounds tend to have much larger volume of distribution and tend to expand when the volume of extracellular water expands greatly, as occurs during the acute inflammatory phase, thus high starting doses may be optimal[12]. On the other hand, for lipophilic agents (as linezolid and macrolides), the dilution in interstitial fluids is less relevant, but they penetrate deeper into fatty tissues and thus, published evidence supports larger doses in patients with a greater amount of adipose tissue[140].

Critically ill patients are predisposed to drug interactions due to the complexity of drug regimens. In critically ill patients, interactions of antimicrobials with other pharmacological classes have been described, including immunosuppressants, statins, benzodiazepins, antipsicotics, antiepileptics, antiarrythmics, loop diuretics and calcium channel blockers[141]. The drug interaction profile of β-lactams is typically associated with the inhibition of their renal secretion while interactions of macrolides and azalides depend on the inhibition of the CYP450 system and P-glycoprotein. Main interactions of aminoglycosides derive from additive or synergistic effects with other drugs for nephrotoxicity, ototoxicity and neuromuscular blockade. For quinolones, in addition to chelation-related interactions, the risk of QTc prolongation implies monitoring in patients with history of QT prolongation or uncorrected electrolyte abnormalities and those receiving antiarrythmics. Few drug interactions have been described for vancomycin (but it should be taken into account its non-negligible nephrotoxicity, that may increase with the concomitant use of aminoglycosides), for daptomycin, linezolid and tigecycline[141].

INFECTIONS IN THE ICU ENVIRONMENT

Hospital-acquired infections affect a quarter of critically ill patients and can double the risk of patient dying[10], with more than one-quarter of all nosocomial infections diagnosed in the ICU[142]. Principal infections diagnosed and/or treated in ICU patients are: respiratory tract infections (nosocomial pneumonia/ventilator-associated pneumonia (VAP)), bloodstream infections (primary bacteremia/catheter-associated bacteremia), urinary tract infections, intraabdominal infections, endocarditis, and surgical wound infections. Table 1 shows by type of infection, microorganisms to be suspected in relation to the presence or not of risk factors for multidrug resistance, and suggested empirical treatments. Table 2 shows recommended antibiotic regimens for critically ill patients.

Respiratory tract infections

Etiology of early-onset infections may be distinguished

from that of late-onset infections. When the disease develops within 4 days of admission or intubation, core organisms are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Moraxella catharralis*, microorganisms associated with community-acquired pneumonia[143]. When the disease develops after 5 days, in addition to these core organisms, enterobacteria (*K. pneumoniae*, *E. coli* and the AmpC-producing microorganisms included in the ESCPM group) and *S. aureus* predominate[143]. These last organisms also predominate in patients with severe comorbidities and recent antimicrobial therapy, thus the distinction between early and late onset is far from absolute. In addition, longer duration of mechanical ventilation and treatments with broad-spectrum antimicrobial therapy increase the risk for *P. aeruginosa, Acinetobacter* spp. and MRSA[143], being enterobacteria and non-fermentative gram-negatives more frequent in VAP vs. non-VAP nosocomial pneumonia[144]. Of relevance is that 20 to 50% of VAP cases have polymicrobial etiology[143], and that ESKAPE organisms (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp.), with their associated resistance profile, constitute 80% of VAP episodes[145].

Bloodstream infections

Critically ill patients carry much higher rates of bloodstream infections than patients in general wards, with an incidence in the ICU ranging from 3 to 10 episodes/100 ICU admissions[146]. Staphylococci seems to predominate both in primary bloodstream infections and in those associated with devices[147-149], and although *S. aureus* is a frequent cause, coagulase-negative staphylococci has become the most common cause in last decades[150]. However, a significant increase in the incidence of bloodstream infections caused by gram-negatives and fungi has been described[151]. In a recent multinational study including 162 ICUs, ≈58% bloodstream infections were caused by gram-negatives, 32.8% by gram-positives, 7.8% by fungi and 1.2% by strict anaerobes[152]. The rate of polymicrobial infections was 12%[152], but in another study in our country the rate was considerably higher (20%)[153]. The increase in the empirical use of broad-spectrum antibiotics has increased the rate of non-classical bacterial isolates as enterobacteria, non-fermenters and fungi in infusion-related and cannula-related infections[150]. Studies in the ICU have shown that *Pseudomonas*, *Acinetobacter* and enterococci in addition to staphylococci (including MRSA) are common cause of bloodstream infections[147,148]. In addition, ESBL-producing *E. coli* should not be forgotten as common cause of nosocomial bloodstream infections[154].

Urinary tract infections (UTIs)

It has been estimated that UTIs represent 20-50% of all ICU infections[155], the majority of them associated with the use of urethral catheters[156]. Duration of catheterization is the main risk factor, with short-term (<30 days) duration associated with a prevalence of 30% and long-term (≥30 days) duration with a 90% prevalence of UTI[157]. *E. coli, Klebsiella*, *Pseudomonas* and enterococci are target bacteria associated with short-term duration of catheterization whereas long-term duration is associated, in addition to the previously cited microorganisms, with members of the ESCPM group (with their AmpC production), and with the possibility of polymicrobial infection[157]. It should be considered that the most frequent source of bacteremia caused by ESBL-producing bacteria was UTI infection in a Dutch multicenter study[158], and that multidrug resistant UTIs may be very frequent among patients with sepsis admitted in the ICU[159].

Intraabdominal infections

Core microorganisms are enterobacteria, as *E. coli* or *K. pneumoniae*, and *Bacteroides* spp. (mainly, *Bacteroides fragilis*) in infections in patients with less than 5 days of hospitalization. There are discussions about the role of *Enterococcus* spp., which in some studies plays a minor role in secondary peritonitis[160] but in others increases the rate of morbidity[161]. In a published study on secondary bacterial peritonitis, higher rates of isolation were found when there was a nosocomial onset of the disease, higher values of Charlson and APACHE II scores, rapidly fatal disease and ICU admission[162]. When the onset of the infection occurs in patients with>5 days of hospitalization, and thus there are risks for infection by multidrug resistant bacteria, in addition to core microorganisms, non-fermentative gram-negatives (*P. aeruginosa*, *Acinetobacter* spp.) and ESBL-producing *E. coli* and *K. pneumoniae* should also be suspected. *P. aeruginosa* is more frequently isolated in intraabdominal infections of nosocomial origin and the frequency of ESBL-producers in intraabdominal infections in a multicenter study in our country was ≈8.5% for *K. pneumoniae* and *E. coli*[83]. Importantly, the second most frequent source of bacteremia caused by ESBL-producing bacteria in the Dutch multicenter study previously commented was intraabdominal infection (after UTI)[158].

Endocarditis

Infective endocarditis still carries high morbidity and mortality for the subset of patients requiring ICU admission. Staphylococci and streptococci account for the majority of cases, with trends towards a rising prevalence of cases by staphylococcal skin flora from nosocomial iatrogenic origin[163]. Common blood cultures in infective endocarditis include *S. aureus* (with special importance in intravenous drug users), viridans streptococci (among them *Streptococcus bovis* in the elderly is often associated with underlying gastrointestinal neoplasm), enterococci and coagulase-negative staphylococci[163-165]. Culture-negative infective endocarditis may be up to one-third cases[166], and the HACEK group (*Haemophilus* spp., *Aggregatibacter* -formerly *Actinobacillus- actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens*, *Kingella* spp.) accounts for 5-10% of all cases of infective endocarditis[167]. Percentages for each etiological agent may differ if endocarditis affects native valves or intracardiac devices. While viridans streptococci is more frequent in native valve endocarditis in non-drug users, coagulase-negative staphy-

Table 1 **By type of infection, microorganisms to be suspected in relation to the presence or not of risk factors for multidrug resistance and suggested empirical treatments [VAP: ventilador-associated pneumonia; MDR: multidrug resistance; ESBL: extended-spectrum β-lactamase; ESCPM group (*Enterobacter cloacae, Enterobacter aerogenes, Serratia marcescens, Citrobacter freundii, Providencia rettgeri* and *Morganella morganii*); MRSA: methicillin-resistant *S. aureus*; HACEK (*Haemophilus* spp., *Aggregatibacter* -formerly *Actinobacillus-actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella* spp)]

INFECTION TYPE	SUSPECTED PATHOGENS	EMPIRICAL TREATMENT	COMMENTS
Pneumonia	**No risk factors for MDR bacteria** *S. pneumoniae* *H. influenzae* *S. aureus* (methicillin-susceptible) *Enterobacteriaceae* *Legionella*	Cefotaxime or ertapenem ± Azithromycin or levofloxacin	IV antibiotic treatment should not exceed >7 days Addition of macrolides/azalides improves the prognosis of pneumococcal pneumonia
	Presence of risk factors for first-level of resistance[1] Above microorganisms plus: ESBL-producing enterobacteria Penicillin-resistant *S. pneumoniae* *P. aeruginosa* MRSA	Piperacillin/tazobactam or cefepime or meropenem or doripenem PLUS Levofloxacin or amikacin ± Linezolid	ESBL-producing isolates are involved in ≈10% pneumonia caused by enterobacteria. When confirmed, monotherapy with carbapenems (meropenem, imipenem, ertapenem) is indicated Suspiction of infection by *P. aeruginosa*: It is recommended the association of two antipseudomonal compounds In bacteremic infections by MRSA, consider the association of linezolid + daptomycin
	Presence of risk factors for second-level of resistance[2] Above microorganisms plus: Non-fermenter gramnegative bacilli AmpC and/or carbapenemase-producing enterobacteria Multidrug-resistant *P. aeruginosa*	Antipseudomonal betalactam different from those previously used, with preference for carbapenems PLUS Levofloxacin or amikacin PLUS Linezolid	Treatment election should consider local epidemiology, previous antibiotic treatments and susceptibility of isolates in surveillance cultures of colonizing flora Consider administration of an inhalated antibiotic Consider associations with colimycin, fosfomycin and tigecycline

Table 1 By type of infection, microorganisms to be suspected in relation to the presence or not of risk factors for multidrug resistance and suggested empirical treatments [VAP: ventilador-associated pneumonia; MDR: multidrug resistance; ESBL: extended-spectrum β-lactamase; ESCPM group (*Enterobacter cloacae, Enterobacter aerogenes, Serratia marcescens, Citrobacter freundii, Providencia rettgeri* and *Morganella morganii*); MRSA: methicillin-resistant *S. aureus*; HACEK (*Haemophilus* spp., *Aggregatibacter* -formerly *Actinobacillus-actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella* spp)] (CONT.)

INFECTION TYPE	SUSPECTED PATHOGENS	EMPIRICAL TREATMENT	COMMENTS
Bloodstream infections: primary bacteremia/ catheter-associated bacteremia	Coagulase-negative staphylococci *S. aureus* (including MRSA) *Enterococcus* spp. *E. coli* *Klebsiella* spp. ESCPM group *P. aeruginosa* *Acinetobacter* spp.	Daptomycin PLUS Cefepime or piperacillin/tazobactam or meropenem or doripenem ± Amikacin	Gram-negative bacteria should always be suspected in the critically ill patient regardless site of central venous catheter If methicillin-susceptibility in staphylococci is confirmed, change to cloxacillin In persistent (>5-7 days) or recurrent (without endovascular foci) bacteremia by *S. aureus*, a second anti-staphylococcal drug (with or without rifampicin) should be added. If the patient is under cloxacillin treatment, add daptomycin with or without rifampicin. If the patient is under daptomycin treatment, add linezolid or fosfomycin or cloxacillin, with or without rifampicin. If the patient is under vancomycin treatment, change to daptomycin + cloxacillin, with or without rifampicin
	Candida spp.	Echinocandin or fluconazol	An antifungal drug with activity against *Candida* spp. should be considered in critically ill patients with central venous catheter in the femoral vein and/or parenteral nutrition, severe sepsis or recent abdominal surgery
Urinary tract infections	**With criteria for severe sepsis or presence of risk factors for first-level of resistance**[1] ESBL-producing enterobacteria	Meropenem or doripenem ± Amikacin	Due to its high frequency, ESBL-producing enterobacteria should be covered in patients with severe sepsis or septic shock
	Presence of risk factors for second-level of resistance[2] Above microorganisms plus:	Meropenem or doripenem + amikacin ± Fluconazol	Treatment election should consider local epidemiology, previous antibiotic treatments and susceptibility of isolates in surveillance cultures of colonizing flora
	ESCPM group Multidrug-resistant *P. aeruginosa*, *Enterococcus* spp. *Acinetobacter* spp. *Candida* spp.		Use of colimycin or tigecycline may be necessary. Although tigecycline concentrations in urine are not high, it may be useful in case of pyelonephritis

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INFECTION TYPE	SUSPECTED PATHOGENS	EMPIRICAL TREATMENT	COMMENTS
Intraabdominal infections	**No risk factors for MDR bacteria** *E. coli* *K. pneumoniae* *B. fragilis*	Ertapenem or cefotaxime + metronidazole	In case of lack of control of the infectious foci, follow treatment recommendations in the presence of risk factors for first-level resistance
	Presence of risk factors for first-level of resistance[1] Above microorganisms plus: ESBL-producing enterobacteria *P. aeruginosa* *Enterococcus* spp. MRSA	Meropenem or imipenem or ertapenem ± Daptomycin or linezolid or vancomycin OR Tigecycline ± Piperacillin/tazobactam or cefepime or amikacin	In case of lack of control of the infectious foci, follow treatment recommendations in the presence of risk factors for second-level resistance
	Presence of risk factors for second-level of resistance[2] All the above microorganisms plus: Non-fermenter gramnegative bacilli AmpC and/or carbapenemase-producing enterobacteria Multidrug-resistant *P. aeruginosa* *Candida* spp.	Meropenem or doripenem + daptomycin or linezolid or vancomycin OR Tigecycline + piperacillin/tazobactam or cefepime ± Amikacin ± Echinocandin	Treatment election should consider local epidemiology, previous antibiotic treatments and susceptibility of isolates in surveillance cultures of colonizing flora In critically ill patients, echinocandins are the elective treatment for *Candida* antifungal therapy

Table 1 **By type of infection, microorganisms to be suspected in relation to the presence or not of risk factors for multidrug resistance and suggested empirical treatments [VAP: ventilador-associated pneumonia; MDR: multidrug resistance; ESBL: extended-spectrum β-lactamase; ESCPM group (*Enterobacter cloacae, Enterobacter aerogenes, Serratia marcescens, Citrobacter freundii, Providencia rettgeri* and *Morganella morganii*); MRSA: methicillin-resistant *S. aureus*; HACEK (*Haemophilus* spp., *Aggregatibacter* -formerly *Actinobacillus-actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella* spp)] (CONT.)**

INFECTION TYPE	SUSPECTED PATHOGENS	EMPIRICAL TREATMENT	COMMENTS
Endocarditis Native valve Prosthetic valve >12 months post-surgery	*S. aureus* Coagulase-negative staphylococci Viridans group streptococci *Enterococcus* spp. *Streptococcus bovis* HACEK group	Ampicillin + cloxacillin ± Gentamicin (3-5 days)	If glomerular filtrate is <40 ml/min or concomitant treatment with potentially neurotoxic drugs, change gentamicin by daptomycin
	Risk for MRSA (including intravenous drug users and healthcare facilities)	Ampicillin + daptomycin + fosfomycin ± Gentamicin (3-5 days) OR Ampicillin + vancomycin	If vancomycin MIC ≥1 mg/L, severe sepsis or bacteremia for >5 days, consider heteroresistance or tolerance and change to daptomycin Addition of gentamin should be avoided if glomerular filtrate is <40 ml/min. Consider change to cotrimoxazole. Addition of fosfomycin should be avoided if MIC ≥32 mg/L. Consider change to cotrimoxazole
Prosthetic valve <12 months post-surgery	MRSA Coagulase-negative staphylococci Viridans group streptococci *Enterococcus* spp. *Streptococcus bovis* HACEK group *E. coli* *K. pneumoniae* *Salmonella enteritidis* *P. aeruginosa*	Daptomycin + rifampicin (3-5 days) ± fosfomycin ± gentamicin or amikacin PLUS Meropenem	Vancomycin could be considered when MIC≤1 mg/L for the MRSA and normal renal function Addition of gentamin should be avoided if glomerular filtrate is <40 ml/min. Consider change to cotrimoxazole. Addition of fosfomycin should be avoided if MIC ≥32 mg/L. Consider change to cotrimoxazole Considerar gentamicin if *Enterococcus* spp. is isolated

Table 1 **By type of infection, microorganisms to be suspected in relation to the presence or not of risk factors for multidrug resistance and suggested empirical treatments [VAP: ventilador-associated pneumonia; MDR: multidrug resistance; ESBL: extended-spectrum β-lactamase; ESCPM group (*Enterobacter cloacae, Enterobacter aerogenes, Serratia marcescens, Citrobacter freundii, Providencia rettgeri* and *Morganella morganii*); MRSA: methicillin-resistant *S. aureus*; HACEK (*Haemophilus* spp., *Aggregatibacter* -formerly *Actinobacillus-actinomycetemcomitans, Cardiobacterium hominis, Eikenella corrodens, Kingella* spp)] (CONT.)**

INFECTION TYPE	SUSPECTED PATHOGENS	EMPIRICAL TREATMENT	COMMENTS
Skin and Soft tissue infections Necrotizing fascitis (Fournier's gangrene, early surgical wound infection 24-48 h post-surgery)	Group A streptococci ***Clostridium perfringes*** ***Clostridium septicum*** ***Staphylococcus aureus*** **Mixed polymicrobial infection:** *Enterococcus* spp. *Bacillus cereus* *E. coli* *P. aeruginosa* *Klebsiella* spp. *Proteus* spp. *Peptostreptococcus* spp. *Bacteroides* spp.	Piperacillin/tazobactam or meropenem PLUS Daptomycin or linezolid or clindamycin OR Tigecycline	In infections by *S. aureus* producing panton valentine leukocidin or superantigens, the antibiotic regimen should include linezolid or clindamicin Consider high doses of tigecycline in moderately severe polymicrobial infections involving MRSA and in patients with allergy to ß-lactams

[1]Risk factors for first-level of resistance: Significant comorbidities and/or antibiotic treatment for >3-5 days

[2]Risk factors for second-level of resistance: Hospital admission and/or prolonged antibiotic treatment (>7 days)

Table 2 **Doses of common antibiotics for the treatment of infections in the critically ill patient**

Drug	Dose (iv)	Comments
Amikacin	20-30 mg/kg / 24 h	
Ampicillin	2 g / 6 h	1-2 g as initial dose followed by 8g in 24h continuous infusion
Azithromycin	500 mg / 24 h	
Cefepime	2 g / 8 h	1-2 g as initial dose followed by 6g in 24h continuous infusion
Ceftazidime	2 g / 8 h	1-2 g as initial dose followed by 6g in 24h continuous infusion
Cefotaxime	2 g / 6-8 h	1-2 g as initial dose followed by 6g in 24h continuous infusion
Ciprofloxacin	400 mg / 8 h	
Cloxacillin	2 g / 4 h	1-2 g as initial dose followed by 12g in 24h continuous infusion
Cotrimoxazole	5 mg/kg of trimetropin / 8 h	
Colimycin	$9x10^6$ U followed by $4.5x10^6$ U / 12 h	
Daptomycin	10 mg/kg/day	May be administered as bolus
Doripenem	1 g / 8 h	Administered as intermittent slow infusion (4 h)
Ertapenem	1 g / 12 h	
Fosfomycin	4-8 g / 8 h	Administered as intermittent slow infusion (4 h) or continuous infusion
Gentamicin	7-9 mg/kg/day (1 dosis)	Referred to adjusted body weigth; body weight = ideal body weight + 0.4 x (total weight –ideal weight)
Imipenem	1 g / 8 h	Intermittent slow infusion (2 h)
Levofloxacin	500 mg / 12 h	
Linezolid	600 mg / 8-12 h	1200 mg in 24 h continuous infusion
Meropenem	2 g / 8 h	Intermittent slow infusion (3 h)
Metronidazole	500 mg / 8 h	
Piperacillin-tazobactam	4-0.5 g / 6 h	2 g as initial dose followed by 16g in 24h continuous infusion
Rifampicin	600 mg / 12-24 h	
Tigecycline	100- 200 mg followed by 50- 100 mg / 12 h	
Vancomycin	15-20 mg/kg / 8 h (in 1-2 h)	Kg referred to total body weight
	35 mg/kg as loading dose followed by 35 mg/kg / day in continuous infusion	

lococci is more frequent in infective endocarditis in patients with intracardiac devices[168] but, in all cases, *S. aureus* is the most frequent pathogen[168].

Surgical wound infections

Bacterial contamination of surgical wounds is inevitable, but common wound pathogens depend on clean / contaminated surgical procedures[169]. For clean surgical procedures, staphylococci are the most common cause of wound infections, and the patient's microbiota has been implicated as the most likely source. *S. aureus* nare colonization appears to be the major risk factor for developing *S. aureus* wound infection. This has particular importance in selected populations where colonization rates exceed 50%, as diabetic individuals and hemodyalized patients[169]. Considering the high rates of methicillin resistance among *S. aureus*, the possibility of infection by MRSA isolates should always be suspected.

For contaminated procedures, wound pathogens frequently are among those species that comprise normal flora of the viscus entered during the surgical procedure. In this sense, polymicrobial infections are common in digestive surgery involving colorectal procedures, with enterobacteria (*E. coli* and *Klebsiella*) and *B. fragilis* as target bacteria. The possibility of a high prevalence of intestinal colonization with ESBL-producing enterobacteria on ICU admission should always be considered in this context[170].

CONCLUSIONS

Antibiotic treatment and use of medical devices are highly frequent in severely ill patients requiring specialized care.

This fact and the concentration of high-risk patients in ICUs constitute accumulative factors for multidrug antibiotic resistance. In hospitals, ICUs are considered areas where antibiotic resistance problems are the largest, and ICU physicians feel that this problem is major and significant in their clinical practice. Recently new antibiotics have been available to overcome non-susceptibility phenotypes in gram-positive microorganisms. However, the plethora of mechanisms of resistance in gram-negative bacteria, new emerging mechanisms and accumulation of resistance traits have lead to multidrug resistance, a worrisome problem in the treatment of gram-negative infections. Intensive care physicians should be aware of the local epidemiology of resistance to select the most appropriate drugs in the antibiotic regimen.

CONFLICT OF INTERESTS

All authors comply with ethical responsibilities and author's requirements, and declare no conflict of interest.

ACKNOWLEDGEMENTS

The authors acknowledge Novartis S.A. for supporting the meeting of the GTIPO-SEDAR held in Valencia in May 2012

REFERENCES

1. **Martínez JL, Baquero F. Interactions among strategies associated with bacterial infection: pathogenicity, epidemicity, and antibiotic resistance. Clin Microbiol Rev 2002;15:647-79.**
2. Baquero F. Environmental stress and evolvability in microbial systems. Clin Microbiol Infect 2009;15 (Suppl 1):5-10.
3. Hawkey PM. The growing burden of antimicrobial resistance. J Antimicrob Chemother 2008;62 (Suppl 1):i1-9.
4. Baquero MR, Galán JC, del Carmen Turrientes M, Cantón R, Coque TM, Martínez JL, et al. Increased mutation frequencies in *Escherichia coli* isolates harboring extended-spectrum beta-lactamases. Antimicrob Agents Chemother 2005;49:4754-6.
5. Ubeda C, Maiques E, Knecht E, Lasa I, Novick RP, Penadés JR. Antibiotic-induced SOS response promotes horizontal dissemination of pathogenicity island-encoded virulence factors in staphylococci. Mol Microbiol 2005;56:836-44.
6. Giménez M, García-Rey C, Barberán J, Aguilar L. Clinical experience with tigecycline in the treatment of nosocomial infections caused by isolates exhibiting prevalent resistance mechanisms. [Article in Spanish]. Rev Esp Quimioter 2009;22:48-56.
7. Graffunder EM, Preston KE, Evans AM, Venezia RA. Risk factors associated with extended-spectrum beta-lactamase-producing organisms at a tertiary care hospital. J Antimicrob Chemother 2005;56:139-45.
8. Lautenbach E, Weiner MG, Nachamkin I, Bilker WB, Sheridan A, Fishman NO. Imipenem resistance among *Pseudomonas aeruginosa* isolates: risk factors for infection and impact of resistance on clinical and economic outcomes. Infect Control Hosp Epidemiol 2006;27:893-900.
9. Martínez JA, Aguilar J, Almela M, Marco F, Soriano A, López F, et al Prior use of carbapenems may be a significant risk factor for extended-spectrum beta-lactamase-producing *Escherichia coli* or *Klebsiella* spp. in patients with bacteraemia. J Antimicrob Chemother 2006;58:1082-5.
10. Vincent JL, Rello J, Marshall J, Silva E, Anzueto A, Martin CD, et al. International study of the prevalence and outcomes of infection in intensive care units. JAMA 2009;302:2323-9.
11. Bueno-Cavanillas A, Delgado-Rodríguez M, López-Luque A, Schaffino-Cano S, Gálvez-Vargas R. Influence of nosocomial infection on mortality rate in an intensive care unit. Crit Care Med 1994;22:55-60.
12. McKenzie C. Antibiotic dosing in critical illness. J Antimicrob Chemother 2011;66 (Suppl 2):ii25-31.
13. Pittet D. Infection control and quality health care in the new millennium. Am J Infect Control 2005;33:258-67.
14. Vincent JL, Sakr Y, Sprung CL, Ranieri VM, Reinhart K, Gerlach H, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med 2006;34:344-53.
15. Malacarne P, Langer M, Nascimben E, Moro ML, Giudici D, Lampati L, et al. Building a continuous multicenter infection surveillance system in the intensive care unit: findings from the initial data set of 9,493 patients from 71 Italian intensive care units. Crit Care Med 2008;36:1105-13.
16. Malacarne P, Boccalatte D, Acquarolo A, Agostini F, Anghileri A, Giardino M, et al. Epidemiology of nosocomial infection in 125 Italian intensive care units. Minerva Anestesiol 2010;76:13-23.
17. Grundmann H, Aires-de-Sousa M, Boyce J, Tiemersma E. Emergence and resurgence of meticillin-resistant *Staphylococcus aureus* as a public-health threat. Lancet 2006;368:874-85.
18. Olaechea PM, Insausti J, Blanco A, Luque P. Epidemiología e impacto de las infecciones nosocomiales [Article in Spanish]. Med Intensiva 2010;34:256-67.
19. Hall IM, Barrass I, Leach S, Pittet D, Hugonnet S. Transmission dynamics of methicillin-resistant *Staphylococcus aureus* in a medical intensive care unit. J R Soc Interface 2012;9:2639-52.
20. Schweickert B, Geffers C, Farragher T, Gastmeier P, Behnke M, Eckmanns T, et al. The MRSA-import in ICUs is an important predictor for the occurrence of nosocomial MRSA cases. Clin Microbiol Infect 2011;17:901-6.
21. Nair N, Kourbatova E, Poole K, Huckabee CM, Murray P, Huskins WC, et al. Molecular epidemiology of methicillin-resistant *Staphylococcus aureus* (MRSA) among patients admitted to adult intensive care units: the STAR*ICU trial. Infect Control Hosp Epidemiol 2011;32:1057-63.
22. Leclercq R. Epidemiological and resistance issues in multidrug-resistant staphylococci and enterococci. Clin Microbiol Infect 2009;15:224-31.
23. Tacconelli E. Antimicrobial use: risk driver of multidrug resistant microorganisms in healthcare settings. Curr Opin Infect Dis 2009;22:352-8.
24. Dancer SJ. The effect of antibiotics on methicillin-resistant *Staphylococcus aureus*. J Antimicrob Chemother 2008;61:246-53.
25. Ginn AN, Wiklendt AM, Gidding HF, George N, O'Driscoll JS, Partridge SR, et al. The ecology of antibiotic use in the ICU: homogeneous prescribing of cefepime but not tazocin selects for antibiotic resistant infection. PLoS One 2012;7:e38719.
26. Appelbaum PC. The emergence of vancomycin-intermediate and

vancomycin-resistant *Staphylococcus aureus*. Clin Microbiol Infect 2006;12 (Suppl 1):16-23.

27. French GL. Bactericidal agents in the treatment of MRSA infections--the potential role of daptomycin. J Antimicrob Chemother 2006;58:1107-17.
28. Howe RA, Monk A, Wootton M, Walsh TR, Enright MC. Vancomycin susceptibility within methicillin-resistant *Staphylococcus aureus* lineages. Emerg Infect Dis 2004;10:855-7.
29. Liu C, Chambers HF. *Staphylococcus aureus* with heterogeneous resistance to vancomycin: epidemiology, clinical significance, and critical assessment of diagnostic methods. Antimicrob Agents Chemother 2003;47:3040-5.
30. Khosrovaneh A, Riederer K, Saeed S, Tabriz MS, Shah AR, Hanna MM, et al. Frequency of reduced vancomycin susceptibility and heterogeneous subpopulation in persistent or recurrent methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis 2004;38:1328-30.
31. Tenover FC, Moellering RC Jr. The rationale for revising the Clinical and Laboratory Standards Institute vancomycin minimal inhibitory concentration interpretive criteria for *Staphylococcus aureus*. Clin Infect Dis 2007;44:1208-15.
32. Campanile F, Borbone S, Perez M, Bongiorno D, Cafiso V, Bertuccio T, et al. Heteroresistance to glycopeptides in Italian meticillin-resistant *Staphylococcus aureus* (MRSA) isolates. Int J Antimicrob Agents 2010;36:415-9.
33. Holmes NE, Johnson PD, Howden BP. Relationship between vancomycin-resistant *Staphylococcus aureus*, vancomycin-intermediate *S. aureus*, high vancomycin MIC, and outcome in serious *S. aureus* infections. J Clin Microbiol 2012;50:2548-52.
34. Aguilar L, Giménez MJ, Barberán J. Glycopeptide heteroresistance and tolerance in hospital grampositive isolates: "invisible" phenomena to the clinician with clinical implications? [Article in Spanish]. Rev Esp Quimioter 2009;22:173-9.
35. Bourgeois I, Pestel-Caron M, Lemeland JF, Pons JL, Caron F. Tolerance to the glycopeptides vancomycin and teicoplanin in coagulase-negative staphylococci. Antimicrob Agents Chemother 2007;51:740-3.
36. Jones RN. Microbiological features of vancomycin in the 21st century: minimum inhibitory concentration creep, bactericidal/static activity, and applied breakpoints to predict clinical outcomes or detect resistant strains. Clin Infect Dis 2006;42 (Suppl 1): S13-24.
37. Gonzalez N, Sevillano D, Alou L, Cafini F, Gimenez MJ, Gomez-Lus ML, et al. Influence of the MBC/MIC ratio on the antibacterial activity of vancomycin versus linezolid against methicillin-resistant *Staphylococcus aureus* isolates in a pharmacodynamic model simulating serum and soft tissue interstitial fluid concentrations reported in diabetic patients. J Antimicrob Chemother 2013; 68:2291-5
38. Traczewski MM, Katz BD, Steenbergen JN, Brown SD. Inhibitory and bactericidal activities of daptomycin, vancomycin, and teicoplanin against methicillin-resistant *Staphylococcus aureus* isolates collected from 1985 to 2007. Antimicrob Agents Chemother 2009;53:1735-8.
39. Alder J, Eisenstein B. The advantage of bactericidal drugs in the treatment of infection. Curr Infect Dis Rep 2004;6:251-3.
40. González C, Rubio M, Romero-Vivas J, González M, Picazo JJ. Bacteremic pneumonia due to *Staphylococcus aureus*: A comparison of disease caused by methicillin-resistant and methicillin-susceptible organisms. Clin Infect Dis 1999;29:1171-7.
41. Cafini F, Aguilar L, González N, Giménez MJ, Torrico M, Alou L, et al. In vitro effect of the presence of human albumin or human serum on the bactericidal activity of daptomycin against strains with the main resistance phenotypes in Gram-positives. J Antimicrob Chemother 2007;59:1185-9.
42. Maor Y, Hagin M, Belausov N, Keller N, Ben-David D, Rahav G. Clinical features of heteroresistant vancomycin-intermediate *Staphylococcus aureus* bacteremia versus those of methicillin-resistant *S. aureus* bacteremia. J Infect Dis 2009;199:619-24.
43. Howden BP, Johnson PD, Ward PB, Stinear TP, Davies JK. Isolates with low-level vancomycin resistance associated with persistent methicillin-resistant *Staphylococcus aureus* bacteremia. Antimicrob Agents Chemother 2006;50:3039-47.
44. Soriano A, Marco F, Martínez JA, Pisos E, Almela M, Dimova VP, et al. Influence of vancomycin minimum inhibitory concentration on the treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. Clin Infect Dis 2008;46:193-200.
45. Picazo JJ, Betriu C, Culebras E, Rodríguez-Avial I, Gómez M, López F, et al. Activity of daptomycin against staphylococci collected from bloodstream infections in Spanish medical centers. Diagn Microbiol Infect Dis 2009;64:448-51.
46. Hidayat LK, Hsu DI, Quist R, Shriner KA, Wong-Beringer A. High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. Arch Intern Med 2006;166:2138-44.
47. Bosso JA, Nappi J, Rudisill C, Wellein M, Bookstaver PB, Swindler J, et al. Relationship between vancomycin trough concentrations and nephrotoxicity: a prospective multicenter trial. Antimicrob Agents Chemother 2011;55:5475-9.
48. Gould IM. The problem with glycopeptides. Int J Antimicrob Agents 2007;30:1-3.
49. Jones RN. Key considerations in the treatment of complicated staphylococcal infections. Clin Microbiol Infect 2008;14 (Suppl 2):3-9.
50. Schramm GE, Johnson JA, Doherty JA, Micek ST, Kollef MH. Methicillin-resistant *Staphylococcus aureus* sterile-site infection: the importance of appropriate initial antimicrobial treatment. Crit Care Med 2006;34:2069–74.
51. Charles PG, Ward PB, Johnson PD, Howden BP, Grayson ML. Clinical features associated with bacteremia due to heterogeneous vancomycin-intermediate *Staphylococcus aureus*. Clin Infect Dis 2004;38:448-51.
52. Howden BP. Recognition and management of infections caused by vancomycin-intermediate *Staphylococcus aureus* (VISA) and heterogenous VISA (hVISA). Intern Med J 2005;35 (Suppl 2):S136-40.
53. Bae IG, Federspiel JJ, Miró JM, Woods CW, Park L, Rybak MJ, et al. Heterogeneous vancomycin-intermediate susceptibility phenotype in bloodstream methicillin-resistant *Staphylococcus aureus* isolates from an international cohort of patients with infective endocarditis: prevalence, genotype, and clinical significance. J Infect Dis 2009;200:1355-66.
54. May J, Shannon K, King A, French G. Glycopeptide tolerance in *Staphylococcus aureus*. J Antimicrob Chemother 1998;42:189-97.
55. Sakoulas G, Moise-Broder PA, Schentag J, Forrest A, Moellering RC Jr, Eliopoulos GM. Relationship of MIC and bactericidal activity to

efficacy of vancomycin for treatment of methicillin-resistant *Staphylococcus aureus* bacteremia. J Clin Microbiol 2004;42:2398-402.
56. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, et al. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006-2007. Infect Control Hosp Epidemiol 2008;29:996-1011.
57. Saribas S, Bagdatli Y. Vancomycin tolerance in enterococci. Chemotherapy 2004;50:250-4.
58. Arias CA, Murray BE. The rise of the *Enterococcus*: beyond vancomycin resistance. Nat Rev Microbiol 2012;10:266-78.
59. Werner G, Coque TM, Hammerum AM, Hope R, Hryniewicz W, Johnson A, et al. Emergence and spread of vancomycin resistance among enterococci in Europe. Euro Surveill 2008;13(47).
60. Sader HS, Watters AA, Fritsche TR, Jones RN. Daptomycin antimicrobial activity tested against methicillin-resistant staphylococci and vancomycin-resistant enterococci isolated in European medical centers (2005). BMC Infect Dis 2007;7:29.
61. Top J, Willems R, Blok H, de Regt M, Jalink K, Troelstra A, et al. Ecological replacement of *Enterococcus faecalis* by multiresistant clonal complex 17 *Enterococcus faecium*. Clin Microbiol Infect 2007;13:316-9.
62. Warren DK, Kollef MH, Seiler SM, Fridkin SK, Fraser VJ. The epidemiology of vancomycin-resistant *Enterococcus* colonization in a medical intensive care unit. Infect Control Hosp Epidemiol 2003;24:257-63.
63. Pan SC, Wang JT, Chen YC, Chang YY, Chen ML, Chang SC. Incidence of and risk factors for infection or colonization of vancomycin-resistant enterococci in patients in the intensive care unit. PLoS One 2012;7:e47297.
64. Batistão DW, Gontijo-Filho PP, Conceição N, Oliveira AG, Ribas RM. Risk factors for vancomycin-resistant enterococci colonisation in critically ill patients. Mem Inst Oswaldo Cruz 2012;107:57-63.
65. Sydnor ER, Perl TM. Hospital epidemiology and infection control in acute-care settings. Clin Microbiol Rev 2011;24:141-73.
66. Rivera AM, Boucher HW. Current concepts in antimicrobial therapy against select gram-positive organisms: methicillin-resistant *Staphylococcus aureus*, penicillin-resistant pneumococci, and vancomycin-resistant enterococci. Mayo Clin Proc 2011;86:1230-43.
67. Donskey CJ, Schreiber JR, Jacobs MR, Shekar R, Salata RA, Gordon S, et al. A polyclonal outbreak of predominantly VanB vancomycin-resistant enterococci in northeast Ohio. Northeast Ohio Vancomycin-Resistant *Enterococcus* Surveillance Program. Clin Infect Dis 1999;29:573-9.
68. Kolar M, Urbanek K, Vagnerova I, Koukalova D. The influence of antibiotic use on the occurrence of vancomycin-resistant enterococci. J Clin Pharm Ther 2006;31:67-72.
69. Song JY, Cheong HJ, Jo YM, Choi WS, Noh JY, Heo JY, et al. Vancomycin-resistant *Enterococcus* colonization before admission to the intensive care unit: a clinico-epidemiologic analysis. Am J Infect Control 2009;37:734-40.
70. Kelesidis T, Tewhey R, Humphries RM. Evolution of high-level daptomycin resistance in *Enterococcus faecium* during daptomycin therapy is associated with limited mutations in the bacterial genome. J Antimicrob Chemother 2013;68:1926-8.
71. Scheetz MH, Knechtel SA, Malczynski M, Postelnick MJ, Qi C. Increasing incidence of linezolid-intermediate or -resistant, vancomycin-resistant *Enterococcus faecium* strains parallels increasing linezolid consumption. Antimicrob Agents Chemother 2008;52:2256-9.
72. Dobbs TE, Patel M, Waites KB, Moser SA, Stamm AM, Hoesley CJ. Nosocomial spread of *Enterococcus faecium* resistant to vancomycin and linezolid in a tertiary care medical center. J Clin Microbiol 2006;44:3368-70.
73. Kainer MA, Devasia RA, Jones TF, Simmons BP, Melton K, Chow S, et al. Response to emerging infection leading to outbreak of linezolid-resistant enterococci. Emerg Infect Dis 2007;13:1024-30.
74. Ntokou E, Stathopoulos C, Kristo I, Dimitroulia E, Labrou M, Vasdeki A, et al. Intensive care unit dissemination of multiple clones of linezolid-resistant *Enterococcus faecalis* and *Enterococcus faecium*. J Antimicrob Chemother 2012;67:1819-23.
75. Moellering RC Jr. The Garrod Lecture. The *Enterococcus*: a classic example of the impact of antimicrobial resistance on therapeutic options. J Antimicrob Chemother 1991;28:1-12.
76. Safdar A, Rolston KV. Vancomycin tolerance, a potential mechanism for refractory gram-positive bacteremia observational study in patients with cancer. Cancer 2006;106:1815-20.
77. Lentino JR, Narita M, Yu VL. New antimicrobial agents as therapy for resistant gram-positive cocci. Eur J Clin Microbiol Infect Dis 2008;27:3-15.
78. Singh SR, Bacon AE 3rd, Young DC, Couch KA. In vitro 24-hour time-kill studies of vancomycin and linezolid in combination versus methicillin-resistant *Staphylococcus aureus*. Antimicrob Agents Chemother 2009;53:4495-7.
79. Steenbergen JN, Mohr JF, Thorne GM. Effects of daptomycin in combination with other antimicrobial agents: a review of in vitro and animal model studies. J Antimicrob Chemother 2009;64:1130-8.
80. Souli M, Galani I, Giamarellou H. Emergence of extensively drug-resistant and pandrug-resistant Gram-negative bacilli in Europe. Euro Surveill 2008; 13 (47). pii: 19045.
81. Livermore DM. Fourteen years in resistance. Int J Antimicrob Agents 2012;39:283-94.
82. Díaz MA, Hernández-Bello JR, Rodríguez-Baño J, Martínez-Martínez L, Calvo J, Blanco J, et al. Diversity of *Escherichia coli* strains producing extended-spectrum beta-lactamases in Spain: second nationwide study. J Clin Microbiol 2010;48:2840-5.
83. Cantón R, Loza E, Aznar J, Calvo J, Cercenado E, Cisterna R, et al. Antimicrobial susceptibility of Gram-negative organisms from intraabdominal infections and evolution of isolates with extended spectrum β-lactamases in the SMART study in Spain (2002-2010). Rev Esp Quimioter 2011;24:223-32.
84. Rubio-Perez I, Martin-Perez E, Garcia DD, Calvo ML, Barrera EL. Extended-spectrum beta-lactamase-producing bacteria in a tertiary care hospital in Madrid: epidemiology, risk factors and antimicrobial susceptibility patterns. Emerg Health Threats J. 2012;5.
85. Paterson DL, Bonomo RA. Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 2005;18:657-86.
86. Hombach M, Mouttet B, Bloemberg GV. Consequences of revised CLSI and EUCAST guidelines for antibiotic susceptibility patterns of ESBL- and AmpC -lactamase-producing clinical *Enterobacteriaceae* isolates. J Antimicrob Chemother. 2013; 68:2092-8.

87. Rodríguez-Baño J, Navarro MD, Romero L, Muniain MA, de Cueto M, Ríos MJ, et al. Bacteremia due to extended-spectrum beta -lactamase-producing *Escherichia coli* in the CTX-M era: a new clinical challenge. Clin Infect Dis 2006;43:1407-14.
88. Dunne WM Jr, Hardin DJ. Use of several inducer and substrate antibiotic combinations in a disk approximation assay format to screen for AmpC induction in patient isolates of *Pseudomonas aeruginosa*, *Enterobacter* spp., *Citrobacter* spp., and *Serratia* spp. J Clin Microbiol 2005;43:5945-9.
89. Livermore DM, Brown DF. Detection of beta-lactamase-mediated resistance. J Antimicrob Chemother 2001;48 (Suppl 1):59-64.
90. Chow JW, Fine MJ, Shlaes DM, Quinn JP, Hooper DC, Johnson MP, et al. *Enterobacter* bacteremia: clinical features and emergence of antibiotic resistance during therapy. Ann Intern Med 1991;115:585-90.
91. Park YS, Yoo S, Seo MR, Kim JY, Cho YK, Pai H. Risk factors and clinical features of infections caused by plasmid-mediated AmpC beta-lactamase-producing *Enterobacteriaceae*. Int J Antimicrob Agents 2009;34:38-43.
92. Livermore DM, Brown DF, Quinn JP, Carmeli Y, Paterson DL, Yu VL. Should third-generation cephalosporins be avoided against AmpC-inducible *Enterobacteriaceae*?. Clin Microbiol Infect 2004;10:84-5.
93. Harris PN, Ferguson JK. Antibiotic therapy for inducible AmpC -lactamase-producing Gram-negative bacilli: what are the alternatives to carbapenems, quinolones and aminoglycosides?. Int J Antimicrob Agents 2012;40:297-305.
94. Mushtaq S, Irfan S, Sarma JB, Doumith M, Pike R, Pitout J, et al. Phylogenetic diversity of *Escherichia coli* strains producing NDM-type carbapenemases. J Antimicrob Chemother 2011;66:2002-5.
95. Palzkill T. Metallo- -lactamase structure and function. Ann N Y Acad Sci 2013;1277:91-104.
96. Wybo I, De Bel A, Soetens O, Echahidi F, Vandoorslaer K, Van Cauwenbergh M, et al. Differentiation of cfiA-negative and cfiA-positive *Bacteroides fragilis* isolates by matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 2011;49:1961-4.
97. Bonomo RA, Szabo D. Mechanisms of multidrug resistance in *Acinetobacter* species and *Pseudomonas aeruginosa*. Clin Infect Dis 2006;43 (Suppl 2):S49-56.
98. Livermore DM. Has the era of untreatable infections arrived?. J Antimicrob Chemother 2009;64 (Suppl 1):i29-36.
99. Walsh TR. Clinically significant carbapenemases: an update. Curr Opin Infect Dis 2008;21:367-71.
100. Suarez C, Peña C, Campo A, Murillas J, Almirante B, Pomar V, et al. Impact of carbapenem resistance on *Pseudomonas aeruginosa* (PA) bloodstream infections outcome. 49th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco 2009; Abstract K-332.
101. Georges B, Conil JM, Dubouix A, Archambaud M, Bonnet E, Saivin S, et al. Risk of emergence of *Pseudomonas aeruginosa* resistance to beta-lactam antibiotics in intensive care units. Crit Care Med 2006;34:1636-41.
102. Girlich D, Naas T, Nordmann P. Biochemical characterization of the naturally occurring oxacillinase OXA-50 of *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 2004;48:2043-8.
103. Lopez-Otsoa F, Gallego L, Towner KJ, Tysall L, Woodford N, Livermore DM. Endemic carbapenem resistance associated with OXA-40 carbapenemase among *Acinetobacter baumannii* isolates from a hospital in northern Spain. J Clin Microbiol 2002;40:4741-3.
104. Da Silva GJ, Quinteira S, Bértolo E, Sousa JC, Gallego L, Duarte A, et al. Long-term dissemination of an OXA-40 carbapenemase-producing *Acinetobacter baumannii* clone in the Iberian Peninsula. J Antimicrob Chemother 2004;54:255-8.
105. Picazo JJ, Betriu C, Rodríguez-Avial I, Culebras E, Gómez M, López F, et al. Antimicrobial resistance surveillance: VIRA STUDY 2006 [Article in Spanish]. Enferm Infecc Microbiol Clin 2006;24:617-28.
106. Kluytmans-Vandenbergh MF, Kluytmans JA, Voss A. Dutch guideline for preventing nosocomial transmission of highly resistant microorganisms (HRMO). Infection 2005;33:309-13.
107. Cisneros JM, Rodríguez-Baño J, Fernández-Cuenca F, Ribera A, Vila J, Pascual A, et al. Risk-factors for the acquisition of imipenem-resistant *Acinetobacter baumannii* in Spain: a nationwide study. Clin Microbiol Infect 2005;11:874-9.
108. del Mar Tomas M, Cartelle M, Pertega S, Beceiro A, Llinares P, Canle D, et al. Hospital outbreak caused by a carbapenem-resistant strain of *Acinetobacter baumannii*: patient prognosis and risk-factors for colonisation and infection. Clin Microbiol Infect 2005;11:540-6.
109. Castanheira M, Mendes RE, Woosley LN, Jones RN. Trends in carbapenemase-producing *Escherichia coli* and *Klebsiella* spp. from Europe and the Americas: report from the SENTRY antimicrobial surveillance programme (2007-09). J Antimicrob Chemother 2011;66:1409-11.
110. Cantón R, Akóva M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin Microbiol Infect 2012;18:413-31.
111. Sánchez-Romero I, Asensio A, Oteo J, Muñoz-Algarra M, Isidoro B, Vindel A, et al. Nosocomial outbreak of VIM-1-producing *Klebsiella pneumoniae* isolates of multilocus sequence type 15: molecular basis, clinical risk factors, and outcome. Antimicrob Agents Chemother 2012;56:420-7.
112. Miró E, Segura C, Navarro F, Sorlí L, Coll P, Horcajada JP, et al. Spread of plasmids containing the bla(VIM-1) and bla(CTX-M) genes and the qnr determinant in *Enterobacter cloacae*, *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolates. J Antimicrob Chemother 2010;65:661-5.
113. Tato M, Coque TM, Ruíz-Garbajosa P, Pintado V, Cobo J, Sader HS, et al. Complex clonal and plasmid epidemiology in the first outbreak of *Enterobacteriaceae* infection involving VIM-1 metallo-beta-lactamase in Spain: toward endemicity?. Clin Infect Dis 2007;45:1171-8.
114. Cendejas E, Gómez-Gil R, Gómez-Sánchez P, Mingorance J. Detection and characterization of *Enterobacteriaceae* producing metallo-beta-lactamases in a tertiary-care hospital in Spain. Clin Microbiol Infect 2010;16:181-3.
115. Curiao T, Morosini MI, Ruiz-Garbajosa P, Robustillo A, Baquero F, Coque TM, et al. Emergence of bla KPC-3-Tn4401a associated with a pKPN3/4-like plasmid within ST384 and ST388 *Klebsiella pneumoniae* clones in Spain. J Antimicrob Chemother 2010;65:1608-14.
116. Solé M, Pitart C, Roca I, Fàbrega A, Salvador P, Muñoz L, et al. First description of an *Escherichia coli* strain producing NDM-1 carbapenemase in Spain. Antimicrob Agents Chemother 2011;55:4402-4.

117. Struelens MJ, Monnet DL, Magiorakos AP, Santos O'Connor F, Giesecke J; European NDM-1 Survey Participants. New Delhi metallo-beta-lactamase 1-producing *Enterobacteriaceae*: emergence and response in Europe. Euro Surveill. 2010;15(46).
118. European Center for Disease Prevention and Control. Antimicrobial resistance surveillance in Europe, 2011. Available at: http://ecdc.europa.eu/en/publications/Publications/antimicrobial-resistance-surveillance-europe-2011.pdf
119. Falagas ME, Rafailidis PI, Kofteridis D, Virtzili S, Chelvatzoglou FC, Papaioannou V, et al. Risk factors of carbapenem-resistant *Klebsiella pneumoniae* infections: a matched case control study. J Antimicrob Chemother 2007;60:1124-30.
120. Patel G, Huprikar S, Factor SH, Jenkins SG, Calfee DP. Outcomes of carbapenem-resistant *Klebsiella pneumoniae* infection and the impact of antimicrobial and adjunctive therapies. Infect Control Hosp Epidemiol 2008;29:1099-106.
121. Gupta N, Limbago BM, Patel JB, Kallen AJ. Carbapenem-resistant *Enterobacteriaceae*: epidemiology and prevention. Clin Infect Dis 2011;53:60-7.
122. Schwaber MJ, Klarfeld-Lidji S, Navon-Venezia S, Schwartz D, Leavitt A, Carmeli Y. Predictors of carbapenem-resistant *Klebsiella pneumoniae* acquisition among hospitalized adults and effect of acquisition on mortality. Antimicrob Agents Chemother 2008;52:1028-33.
123. Daikos GL, Petrikkos P, Psichogiou M, Kosmidis C, Vryonis E, Skoutelis A, et al. Prospective observational study of the impact of VIM-1 metallo-beta-lactamase on the outcome of patients with *Klebsiella pneumoniae* bloodstream infections. Antimicrob Agents Chemother 2009;53:1868-73.
124. Dellinger RP, Levy MM, Rhodes A, Annane D, Gerlach H, Opal SM, et al. Surviving Sepsis Campaign: international guidelines for management of severe sepsis and septic shock, 2012. Intensive Care Med 2013;39:165-228.
125. Martinez MN, Papich MG, Drusano GL. Dosing regimen matters: the importance of early intervention and rapid attainment of the pharmacokinetic/pharmacodynamic target. Antimicrob Agents Chemother 2012;56:2795-805.
126. Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica. Procedimientos en Microbiología Clínica. Análisis farmacocinéticofarmacodinámico en Microbiología: herramienta para evaluar el tratamiento antimicrobiano http://www.seimc.org/documentos/protocolos/microbiologia/
127. Scaglione F, Paraboni L. Pharmacokinetics/pharmacodynamics of antibacterials in the Intensive Care Unit: setting appropriate dosing regimens. Int J Antimicrob Agents 2008;32:294-301.
128. Alou L, Aguilar L, Sevillano D, Giménez MJ, Echeverría O, Gómez-Lus ML, et al. Is there a pharmacodynamic need for the use of continuous versus intermittent infusion with ceftazidime against *Pseudomonas aeruginosa*? An in vitro pharmacodynamic model. J Antimicrob Chemother 2005;55:209-13.
129. Lorente L, Jiménez A, Martín MM, Iribarren JL, Jiménez JJ, Mora ML. Clinical cure of ventilator-associated pneumonia treated with piperacillin/tazobactam administered by continuous or intermittent infusion. Int J Antimicrob Agents 2009;33:464-8.
130. Lorente L, Lorenzo L, Martín MM, Jiménez A, Mora ML. Meropenem by continuous versus intermittent infusion in ventilator-associated pneumonia due to gram-negative bacilli. Ann Pharmacother 2006;40:219-23.
131. Rello J, Sole-Violan J, Sa-Borges M, Garnacho-Montero J, Muñoz E, Sirgo G, et al. Pneumonia caused by oxacillin-resistant *Staphylococcus aureus* treated with glycopeptides. Crit Care Med 2005;33:1983-7.
132. DiMondi VP, Rafferty K. Review of continuous-infusion vancomycin. Ann Pharmacother 2013;47:219-27.
133. Spapen HD, Janssen van Doorn K, Diltoer M, Verbrugghe W, Jacobs R, Dobbeleir N, et al. Retrospective evaluation of possible renal toxicity associated with continuous infusion of vancomycin in critically ill patients. Ann Intensive Care 2011;1:26.
134. Rayner CR, Forrest A, Meagher AK, Birmingham MC, Schentag JJ. Clinical pharmacodynamics of linezolid in seriously ill patients treated in a compassionate use programme. Clin Pharmacokinet 2003;42:1411-23.
135. Buerger C, Plock N, Dehghanyar P, Joukhadar C, Kloft C. Pharmacokinetics of unbound linezolid in plasma and tissue interstitium of critically ill patients after multiple dosing using microdialysis. Antimicrob Agents Chemother 2006;50:2455-63.
136. Adembri C, Fallani S, Cassetta MI, Arrigucci S, Ottaviano A, Pecile P, et al. Linezolid pharmacokinetic/pharmacodynamic profile in critically ill septic patients: intermittent versus continuous infusion. Int J Antimicrob Agents 2008;31:122-9.
137. Ratjen F, Rietschel E, Kasel D, Schwiertz R, Starke K, Beier H, et al. Pharmacokinetics of inhaled colistin in patients with cystic fibrosis. J Antimicrob Chemother 2006;57:306-11.
138. Michalopoulos AS, Falagas ME. Colistin: recent data on pharmacodynamics properties and clinical efficacy in critically ill patients. Ann Intensive Care 2011;1:30.
139. Pea F, Viale P, Furlanut M. Antimicrobial therapy in critically ill patients: a review of pathophysiological conditions responsible for altered disposition and pharmacokinetic variability. Clin Pharmacokinet 2005;44:1009-34.
140. Erstad BL. Dosing of medications in morbidly obese patients in the intensive care unit setting. Intensive Care Med 2004;30:18-32.
141. Pereira JM, Paiva JA. Antimicrobial drug interactions in the critically ill patients. Curr Clin Pharmacol 2013;8:25-38.
142. Palomar M, Vaque J, Alvarez Lerma F, Pastor V, Olaechea P, Fernández-Crehuet J. Nosocomial infection indicators [Article in Spanish]. Med Clin (Barc) 2008;131 (Suppl 3):48-55.
143. Strausbaugh LJ. Nosocomial respiratory infections. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, Bennett's Principles and Practice of Infectious Diseases, 6th ed.. Philadelphia: Elsevier Inc. 2005; 3362-3370.
144. Esperatti M, Ferrer M, Theessen A, Liapikou A, Valencia M, Saucedo LM, et al. Nosocomial pneumonia in the intensive care unit acquired by mechanically ventilated versus nonventilated patients. Am J Respir Crit Care Med 2010;182:1533-9.
145. Sandiumenge A, Rello J. Ventilator-associated pneumonia caused by ESKAPE organisms: cause, clinical features, and management. Curr Opin Pulm Med 2012;18:187-93.
146. Maseda Garrido E, Alvarez J, Garnacho-Montero J, Jerez V, Lorente L, Rodríguez O. Update on catheter-related bloodstream infections in ICU patients. Enferm Infecc Microbiol Clin 2011;29 (Suppl 4):10-5.
147. Olaechea PM, Palomar M, Álvarez-Lerma F, Otal JJ, Insausti J, López-Pueyo MJ, et al. Morbidity and mortality associated with primary and catheter-related bloodstream infections in critically ill patients. Rev Esp Quimioter 2013;26:21-9.

148. Orsi GB, Franchi C, Marrone R, Giordano A, Rocco M, Venditti M. Laboratory confirmed bloodstream infection aetiology in an intensive care unit: eight years study. Ann Ig 2012;24:269-78.
149. Diekema DJ, Beekmann SE, Chapin KC, Morel KA, Munson E, Doern GV. Epidemiology and outcome of nosocomial and community-onset bloodstream infection. J Clin Microbiol 2003;41:3655-60.
150. Beekmann SE, Henderson DK. Infections caused by percutaneous intravascular devices. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, Bennett's Principles and Practice of Infectious Diseases, 6th ed.. Philadelphia: Elsevier Inc. 2005; 3347-3362.
151. Rodríguez-Créixems M, Muñoz P, Martín-Rabadán P, Cercenado E, Guembe M, Bouza E. Evolution and aetiological shift of catheter-related bloodstream infection in a whole institution: the microbiology department may act as a watchtower. Clin Microbiol Infect 2012;19:845-51.
152. Tabah A, Koulenti D, Laupland K, Misset B, Valles J, Bruzzi de Carvalho F, et al. Characteristics and determinants of outcome of hospital-acquired bloodstream infections in intensive care units: the EUROBACT International Cohort Study. Intensive Care Med 2012;38:1930-45.
153. Sancho S, Artero A, Zaragoza R, Camarena JJ, González R, Nogueira JM. Impact of nosocomial polymicrobial bloodstream infections on the outcome in critically ill patients. Eur J Clin Microbiol Infect Dis 2012;31:1791-6.
154. Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Cisneros JM, Peña C, et al. Risk factors and prognosis of nosocomial bloodstream infections caused by extended-spectrum-beta-lactamase-producing *Escherichia coli*. J Clin Microbiol 2010;48:1726-31.
155. López MJ, Cortés JA. Urinary tract colonization and infection in critically ill patients. Med Intensiva 2012;36:143-51.
156. Richards MJ, Edwards JR, Culver DH, Gaynes RP. Nosocomial infections in medical intensive care units in the United States. National Nosocomial Infections Surveillance System. Crit Care Med 1999;27:887-92.
157. Warren JW. Nosocomial urinary tract infections. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, Bennett's Principles and Practice of Infectious Diseases, 6th ed.. Philadelphia: Elsevier Inc. 2005; 3370-3381.
158. Frakking FN, Rottier WC, Dorigo-Zetsma JW, van Hattem JM, van Hees BC, Kluytmans JA, et al. Appropriateness of empirical treatment and outcome in bacteremia caused by extended-spectrum ß-lactamase producing bacteria. Antimicrob Agents Chemother 2013; 57:3092-9.
159. Mojtahedzadeh M, Panahi Y, Fazeli MR, Najafi A, Pazouki M, Navehsi BM, et al. Intensive care unit-acquired urinary tract infections in patients admitted with sepsis: etiology, risk factors, and patterns of antimicrobial resistance. Int J Infect Dis 2008;12:312-8.
160. Röhrborn A, Wacha H, Schöffel U, Billing A, Aeberhard P, Gebhard B, et al. Coverage of enterococci in community acquired secondary peritonitis: results of a randomized trial. Surg Infect (Larchmt) 2000;1:95-107.
161. Gauzit R, Péan Y, Barth X, Mistretta F, Lalaude O; Top Study Team. Epidemiology, management, and prognosis of secondary non-postoperative peritonitis: a French prospective observational multicenter study. Surg Infect (Larchmt) 2009;10:119-27.
162. Cercenado E, Torroba L, Cantón R, Martínez-Martínez L, Chaves F, García-Rodríguez JA, et al. Multicenter study evaluating the role of enterococci in secondary bacterial peritonitis. J Clin Microbiol 2010;48:456-9.
163. Fariñas MC, Llinares P, Almirante B, Barberán J, de Dios Colmenero J, Garau J, et al. New trends in infective endocarditis. Enferm Infecc Microbiol Clin 2011;29 (Suppl 4):22-35.
164. Fernández Guerrero ML, Goyenechea A, Verdejo C, Roblas RF, de Górgolas M. Enterococcal endocarditis on native and prosthetic valves: a review of clinical and prognostic factors with emphasis on hospital-acquired infections as a major determinant of outcome. Medicine (Baltimore) 2007;86:363-77.
165. Pierce D, Calkins BC, Thornton K. Infectious endocarditis: diagnosis and treatment. Am Fam Physician 2012;85:981-6.
166. Naber CK, Erbel R. Infective endocarditis with negative blood cultures. Int J Antimicrob Agents 2007;30 (Suppl 1):S32-6.
167. Tornos P, Gonzalez-Alujas T, Thuny F, Habib G. Infective endocarditis: the European viewpoint. Curr Probl Cardiol 2011;36:175-222.
168. Murdoch DR, Corey GR, Hoen B, Miró JM, Fowler VG Jr, Bayer AS, et al. Clinical presentation, etiology, and outcome of infective endocarditis in the 21st century: the International Collaboration on Endocarditis-Prospective Cohort Study. Arch Intern Med 2009;169:463-73.
169. Talbot TR, Kaiser AB. Post-operative infections and antimicrobial prophylaxis. In: Mandell GL, Bennett JE, Dolin R, eds. Mandell, Douglas, Bennett's Principles and Practice of Infectious Diseases, 6th ed.. Philadelphia: Elsevier Inc. 2005; 3533-3547.
170. Razazi K, Derde LP, Verachten M, Legrand P, Lesprit P, Brun-Buisson C. Clinical impact and risk factors for colonization with extended-spectrum β-lactamase-producing bacteria in the intensive care unit. Intensive Care Med 2012;38:1769-78.