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SPECIAL ARTICLE

Resistance to β-lactams in enterococci

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Abstract Enterococci are intrinsically resistant to several antimicrobial classes and show a great ability to acquire new mechanisms of resistance. Resistance to β-lactam antibiotics is a major concern because these drugs either alone or in combination are commonly used for the treatment of enterococcal infections. Ampicillin resistance, which is rare in *Enterococcus faecalis*, occurs in most of the hospital-associated *Enterococcus faecium* isolates. High-level resistance to ampicillin in *E. faecium* is mainly due to the enhanced production of PBP5 and/or by polymorphisms in the beta subunit of this protein. The dissemination of high-level ampicillin resistance can be the result of both clonal spread of strains with mutated *pbp5* genes and horizontal gene transfer.

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Resistencia a los β-lactámicos en enterococos

Resumen Los enterococos son intrínsecamente resistentes a varias clases de antimicrobianos y presentan una gran capacidad para adquirir mecanismos de resistencia. La resistencia a los antibióticos β-lactámicos es preocupante porque estos fármacos solos o combinados se usan comúnmente para el tratamiento de las infecciones enterocócicas. La mayoría de los aislamientos hospitalarios de *Enterococcus faecium* presentan resistencia a la ampicilina, la cual es rara en *Enterococcus faecalis*. El alto nivel de resistencia a la ampicilina en *E. faecium* se debe principalmente a la hiperproducción de PBP5 y/o a polimorfismos en la subunidad beta de esta proteína. La propagación de esta resistencia puede deberse tanto a la diseminación clonal de cepas con genes *pbp5* mutados como a la transferencia horizontal de genes.

Introduction

Enterococci are major nosocomial pathogens due to their intrinsic resistance to many antimicrobials as well as to their ability to acquire new mechanisms of resistance. Among enterococcal species, *Enterococcus faecalis* and *Enterococcus faecium* are the most frequently encountered[22].

All enterococci exhibit decreased susceptibility to penicillin and ampicillin, as well as high-level resistance to most cephalosporins and all semi-synthetic penicillins, as the result of expression of low-affinity penicillin-binding proteins[16].

For many strains, their level of resistance to ampicillin does not preclude the clinical use of this agent. In fact, ampicillin remains the treatment of choice for enterococcal infections.

Healthcare-associated enterococcal infections caused by *E. faecium* have been increasing significantly over the last decades. This increase began with the emergence of high-level ampicillin resistance followed by the addition of vancomycin resistance; these resistances are very uncommon (and have not been reported together) in *E. faecalis*.

The aim of the present review is to describe the mechanisms and the prevalence of β-lactam resistance in *E. faecalis* and *E. faecium*, since in other species of enterococci such as *E. casseliflavus*, *E. gallinarum*, *E. raffinosus*, *E. avium*, *E. caccae*, *E. dispar*, *E. durans*, *E. hirae*, and others, also of clinical interest in humans although of less frequent presentation, high-level resistance to penicillin and aminopenicillins have not yet been described.

Intrinsic low-level β-lactam resistance

Antibiotics in the β-lactam family inhibit bacterial growth by serving as suicide substrates for the D,D-transpeptidases (also known as penicillin-binding proteins, or PBPs) that catalyze the cross-linking of peptidoglycan peptide side chains during the synthesis of mature peptidoglycan. Once modified by a β-lactam antibiotic, PBPs are inactivated, thereby preventing continued cell wall synthesis[30].

Enterococci express a low-affinity PBP5 (PBP5 in *E. faecium*, and in *E. faecalis* sometimes denominated PBP4) that bind weakly to β-lactam antibiotics. Even strains isolated from primitive populations in the Solomon Islands (who had had little or no exposure to manufactured antibiotics) as well as strains isolated early in the antibiotic era display this property[22]. In general enterococci are approximately 100-fold less susceptible to β-lactams than streptococci[30]. This trait is encoded by chromosomal determinants in the core genome of these organisms and involves the production of the low-affinity class B penicillin-binding protein 5 (PBP5). Due to its relatively low affinity for β-lactams, PBP5 is capable of carrying out peptidoglycan synthesis at concentrations of β-lactam antibiotics that saturate all the transpeptidase domain of other enterococcal PBPs[16].

The level of intrinsic resistance differs among the β-lactam antimicrobials. Generally, penicillins (*e.g.* ampicillin) have the highest activity, carbapenems slightly lower and cephalosporins have the lowest activity[22].

Acquired high-level resistance

High-level resistance to ampicillin emerged in hospitals in the United States during the 1970s and the 1980s[2].

Ampicillin resistance, is rare in *E. faecalis*, but occurs in ∼90% of modern-day hospital-associated *E. faecium* isolates[2,4]. In Argentina, according to Latin American Surveillance of Antimicrobial Resistance (ReLAVRA) data from 2015, ampicillin resistance rate is 1.8% in *E. faecalis* and 85% in *E. faecium*[27].

High-level resistance to ampicillin in *E. faecium* (MIC $\geq$ 128 mg/l) was initially explained by either the enhanced production of PBP5, and/or by polymorphisms in the beta subunit of this protein[8]. Further analysis of *E. faecium* strains with different levels of ampicillin susceptibility revealed that the variability of PBP5 sequences is mostly due to changes in 21 specific positions of the protein, suggesting that a sequential acquisition of mutations could have contributed to the progressive resistance to ampicillin from the early 1980s[9,25].

Another β-lactam resistance mechanism that does not involve PBPs has been detected in an ampicillin resistant laboratory mutant strain of *E. faecium* (MIC > 2000 mg/l). A bypass of the reaction of D,D-transpeptidation that occurs

during the final steps of the peptidoglycan synthesis was detected by removal of the PBP5 gene[19]. Mutations in genes encoding other species-specific proteins that participate in the cell wall synthesis, may also slightly increase the MIC values even in the absence of PBP5[28].

The first isolates of β-lactamase-producing *E. faecalis* were identified in Texas in 1981[22]. Although rare, these isolates have been spread from seven states of the USA[23], and also were described in Lebanon, Canada, and Argentina, where 6 strains were isolated at the Hospital Garrahan[24]. This mechanism of resistance is more common in *E. faecalis* than in *E. faecium*. However, β-lactamase producer *E. faecium* strains were reported, only one isolate in Richmond in 1992 and 8 epidemiologically unrelated isolates in Modena in 2010[29].

Plasmid-mediated *bla* genes (encoding β-lactamases) were first described in *E. faecalis* in 1983[15]. Some strains of *Enterococcus* produce a β-lactamase identical to the staphylococcal enzyme. These strains are characterized by being resistant to penicillin, aminopenicillins (ampicillin) and ureido-penicillins (piperacillin), but susceptible to imipenem and combinations of β-lactams with β-lactamase inhibitors (such as ampicillin–sulbactam), so they no constitute a therapeutic challenge.

Enterococci produce so little β-lactamase that it cannot be detected by routine susceptibility laboratory tests, such as diffusion or dilution methods. These isolates can be detected using nitrocefin, but are so rare that its use is not recommended of routine. They can also be detected by difference in size between the inhibition zone of ampicillin and ampicillin–sulbactam, considering as positive result when difference is greater than 4 mm[17].

Origin and evolution of β-lactam resistance in *E. faecium*

E. faecium population biology is dominated by two main phylogenomic groups, clade A and clade B. Most isolates resistant to ampicillin belong to clade A or ''hospital-associated clade'' mainly comprising *E. faecium* from hospitalized patients[9]. A subgroup within the clade A, clade A1, is enriched in mobile genetic elements and have enhanced ability to colonize and persist in human hosts due to the presence of adhesins and specific metabolic traits. In contrast, the clade B or ''community-associated *E. faecium*'' mostly comprises ampicillin susceptible isolates from healthy, non-hospitalized individuals[14].

Polymorphisms in the PBP5 protein sequences allowed grouping of PBP5 variants in clusters that mimic the phylogenomic diversification of *E. faecium* suggested a model for evolvability of this enterococcal species consisting of a first split of ''clade B'' and ''clade A'' coincidental with human and animal co-habitation occurring 30 000 years ago, and a further split of ''clade A'' in subclades ''A1'' and ''A2'' after the introduction of antibiotics in the late 1940s[9].

Interpretation of susceptibility tests

According to CLSI recommendations, the results of ampicillin susceptibility tests should be used to predict the activity of amoxicillin. Susceptibility to amoxicillin–clavulanate, ampicillin–sulbactam, piperacillin, and piperacillin–tazobactam among non-β-lactamase producing enterococci may be predicted by ampicillin results. Additionally, ampicillin susceptibility can be used to predict imipenem susceptibility, providing the species is confirmed to be *E. faecalis*.

Until recently it was assumed that *E. faecalis* susceptible to ampicillin were also susceptible to penicillin, but in recent years clinical penicillin-resistant but ampicillin-susceptible *E. faecalis* isolates exhibiting an unusual phenotype were reported. Isolates with this phenotype were described in a study performed in Greece between 2003 and 2004, where 90 of 287 isolates of *E. faecalis* presented this phenotype[20]. In a study conducted in Denmark in 2007, 20 of 33 isolates from blood of patients with bacteraemia from 7 hospitals presented this phenotype, being two of them also resistant to imipenem[13]. In another study performed in Brazil between 2006 and 2010, 34 of 317 isolates presented this phenotype associated with resistance to piperacillin[5]. A recent study showed that a point mutation in the PBP4 is responsible to this phenotype[6].

Treatment of enterococcal infections

Uncomplicated enterococcal infections may be adequately treated with monotherapy, whereas for the treatment of severe infections such as endocarditis a synergistic regimen is needed[15].

Penicillin (or ampicillin) alone or combined with an aminoglycoside was for over half a century the treatment of choice for enterococcal infections. Despite the good inhibitory in vitro activity of ampicillin and penicillin against most *E. faecalis*, it has been shown that β-lactam monotherapy is not suitable for treating endovascular infections. These infections usually require bactericidal therapy that, for many strains, is not achieved with the use of ampicillin or penicillin alone as a result of the tolerance of enterococci to these compounds[1].

Enterococci are tolerant to the (normally) bactericidal activity of cell-wall active agents, such as β-lactam antibiotics and vancomycin. Tolerance implies that the bacteria can be inhibited by clinically achievable concentrations of the antibiotic, but will only be killed by concentrations far in excess of the inhibitory concentration. Enterococcal tolerance can be overcome by combining cell-wall active agents with an aminoglycoside[16]. The synergistic effect occurs because the antibiotic that acts on the cell wall allows the aminoglycoside to reach its action site.

In a study performed in Argentina by Predari et al., 201 strains of *E. faecalis* were studied. Penicillin and ampicillin killing-curves were performed with 5 aminoglycosides in 31 selected isolates and it was determined that a synergistic combination was not always achieved even with low levels of resistance, less than 2000 mg/l at that time[26].

In recent years resistance to aminoglycosides increased significantly by the wide spread of genes encoding aminoglycoside modifying enzymes. In addition, the use of aminoglycosides is limited in critically ill patients because of its nephrotoxic effect. The combination of ceftriaxone (or cefotaxime) and ampicillin was recently tested as an alternative.

There are several studies that support the use of combined therapy to treat enterococcal endocarditis infections. Mainardi and colleagues demonstrated that, at low amoxicillin concentrations, the PBPs 4 and 5 would be partially saturated, but the nonessential PBPs 2 and 3 could participate in building the cell wall. The combination with cefotaxime would totally saturate PBPs 2 and 3, producing the bactericidal synergistic effect[18].

A work performed in Argentina to evaluate in vitro activity of ampicillin–ceftriaxone against 30 strains of *E. faecalis* recovered from invasive infections in patients admitted to a universitary hospital, showed synergy in the 73.3% of the isolates[3].

Thereafter, Gavaldà and colleagues showed that the combination of ampicillin plus ceftriaxone was as effective as ampicillin plus gentamicin for the treatment of experimental endocarditis due to non-high-level resistance to aminoglycosides (HLAR) *E. faecalis* and more effective than ampicillin alone in experimental endocarditis due to HLAR *E. faecalis*[10,11]. In a multicenter study conducted by the same group in 13 Spain hospitals, from 43 patients with *E. faecalis* infective endocarditis (21 HLAR and 22 non-HLAR), the cure occurred in 71.4 and 72.7% of patients respectively, at the end of treatment, with only 2 relapses in the non-HLAR *E. faecalis* endocarditis group[12]. Additionally, in 2008 a successful treatment of relapse of a patient unsuccessfully treated with ampicillin-gentamicin was demonstrated[21]. Based on these studies the American Heart Association recommended the use of ampicillin–ceftriaxone as an alternative for the treatment of *E. faecalis* infective endocarditis with HLAR.

A study performed by Fernandez Hidalgo in 2013 compared the effectiveness of ampicillin–ceftriaxone and ampicillin–gentamicin combinations for treating *E. faecalis* infective endocarditis. The study included 291 patients from 17 hospitals in Spain and 1 in Italy and demonstrated that the treatment with ampicillin-ceftriaxone was similar to treatment with ampicillin–gentamicin, with the advantage of this latter combination regarding toxicity[7].

Conclusion

The treatment of serious infections caused by enterococci is a therapeutic challenge due to the ability they have to develop resistance to antibiotics. However, β-lactam antibiotics, alone or in combination, continue to be useful in the treatment of severe enterococcal infections.

Conflict of interest

The authors declare that they have no conflicts of interest.

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