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SPECIAL ARTICLE

Susceptibility to β-lactams in β-hemolytic streptococci

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Abstract Group A (GAS), B (GBS), c (GCS) and G (GGS) β-hemolytic streptococci are important human pathogens. They cause infections of different severity and frequency. Nowadays, after 70 years of use, penicillin is still universally active against GAS, GCS and GGS. However, therapeutic failures have been recorded in 2–28% of pharyngitis cases (median: 12%) attributable to different causes. By contrast, some GBS with reduced susceptibility to penicillin have been described, especially in Japan. In this group of bacteria, it is important to highlight that confirmation by reference methods is mandatory when decreased susceptibility to penicillin is suspected as well as checked for the detection of the mechanisms involved.

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PALABRAS CLAVE
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Sensibilidad a los β-lactámicos en estreptococos β-hemolíticos

Resumen Los estreptococos β-hemolíticos de los grupos A (GAS), B (GBS), C (GCS) y G (GGS) son importantes patógenos humanos. Ellos producen infecciones de diversa gravedad y frecuencia. Aún después de más de 70 años de uso, la penicilina sigue siendo activa *in vitro* frente al 100% de los GAS, GCS y GGS. No obstante se han producido fallas terapéuticas entre el 2-28% de los casos de faringitis (media: 12%), atribuibles a diversas causas. En cambio se han descrito aislados de GBS con sensibilidad reducida a la penicilina, especialmente en Japón. Es importante que toda sospecha de sensibilidad disminuida a la penicilina en este grupo de bacterias sea confirmada por los métodos de referencia y comprobada mediante la detección de los mecanismos involucrados.

Introduction

Group A (*Streptococcus pyogenes*), and large colony group C and G (*Streptococcus dysgalactiae* subsp. *equisimilis*) streptococci share the profile of being transient colonizers of human skin and mucous and produce several infections such as pharyngitis, scarlet fever, acute otitis media, impetigo, erysipelas, cellulitis, necrotizing fasciitis, septic arthritis, pneumonia, bacteremia and toxic shock syndrome, among the most frequent ones. Their impact is not only quantitative (more than 600 million infections annually) but it is also qualitative, regarding the severity of necrotizing fasciitis, myositis, puerperal sepsis, and the toxic shock syndrome. Additionally, group A streptococci (GAS) have been investigated for their significant role in the development of post-streptococcal infection sequelae, including acute rheumatic fever, acute glomerulonephritis, and reactive arthritis. Group A streptococcal infections have also been associated with Tourette's syndrome, tics, and movement and attention deficit disorders[5].

Streptococcus agalactiae or group B streptococci (GBS) colonize the genital and gastrointestinal tract of women and men and vertical transmission from a colonized mother to her newborn during labor can result in life threatening infections. Because of the latter, GBS is the leading cause of neonatal sepsis and meningitis. In addition, it has been recognized as an important pathogen in non-pregnant individuals, especially elderly people and those suffering from underlying medical disorders[29].

Treatment of different streptococcal infections is mainly based on the use of penicillin V administered orally, intramuscular benzathine penicillin, parenteral penicillin G, amoxicillin or cephalosporins (cephalexin, cefotaxime, ceftriaxone). In allergic patients or special clinical presentations, the first options are macrolides (erythromycin, azithromycin, clarithromycin) and/or lincosamides (lincomycin, clindamycin). Clindamycin is the recommended antibiotic to treat severe infections of skin and soft tissues (necrotizing fasciitis, toxic shock syndrome).

Depending on the susceptibility tests, the patient may be treated with tetracyclines (tetracycline, minocycline, doxycycline), vancomycin or fluoroquinolones (levofloxacin, moxifloxacin). Aminoglycosides were added to β-lactam antibiotics to treat rare cases of endocarditis due to groups C or G streptococci.

The aim of the present review is to describe the mechanisms and the prevalence of β-lactam resistance in β-hemolytic streptococci of groups A, B, C and G.

Resistance to β-lactam antibiotics in groups A, C and G streptococci

To date, no group A, C or G streptococci with diminished susceptibility to penicillin or third generation cephalosporins have been detected[31]. Values of minimal inhibitory concentration (MIC), MIC_{90}, published in different studies showed a median of 0.016 μg/ml and a range of 0.0025–0.032 μg/ml. Some authors described ranges of MICs showing some values higher than the CLSI breakpoint for penicillin (0.125 μg/ml) for group C and G streptococci; however, these results were not confirmed by reference centers.

In Japan, 61.4% of intermediate susceptibility (MIC = 0.25 μg/ml) and 2.3% of resistance to penicillin (one isolate with a MIC = 2 μg/ml) was reported in patients with an initial episode of pharyngitis due to *S. pyogenes*; however, these results were also not confirmed[23].

With regard to group A streptococci, one hundred and thirty-three strains were collected in the Rockefeller University (New York, USA) during 80 years and neither of them showed changes in penicillin susceptibility. The MIC_{90} for the oldest strains (0.032 μg/ml) was the same of those collected in the last year of the study[17].

The question of why GAS remains susceptible to penicillin, can only be answered with speculations[8]:

a. β-Lactamase may not be expressed or may be toxic to GAS.
b. Low affinity PBPs are neither expressed nor render organisms nonviable.
c. GAS produce at least four different DNases that could limit the opportunity for acquisition of exogenous DNA via transformation.
d. Circumstances favorable for the development of resistance have not yet occurred.

In spite of its universal susceptibility, the success of pharyngitis treatments with penicillin is not 100%; however it is between 62% and 98% in different series, with a median failure of around 12%[18].

Failures may be due to different causes:

(a) Coexistence of oropharyngeal β-lactamase-producing bacteria

In the oropharynx there are bacteria that degrade penicillin because they produce extracellular β-lactamases (*Staphylococcus aureus*, anaerobes), thus protecting GAS from penicillin[2].

(b) Elimination of *Streptococcus salivarius*

S. salivarius interferes with *S. pyogenes* by means of its bacteriocins. Penicillin eliminates both organisms. As this antibiotic does not preserve *S. salivarius* in its original niche, it does not provide protection from reinfection with GAS[2].

(c) Tolerance

Tolerance is the property of some bacteria by which they evade the bactericidal activity of a specific antibiotic. Several authors have published that the rate of tolerance of *S. pyogenes* and other β-hemolytic streptococci is ≥10%. However, there is insufficient data to support a correlation between tolerance to penicillin and treatment failure, either in clinical cases or in animal studies[32].

Moreover, it has not been demonstrated that an antibiotic should have bactericidal effect to eradicate GAS from the throat. Furthermore, in one study it has been reported that none of the 28 cases of therapeutic failure with penicillin the bacteria showed the tolerance phenomenon[30].

(d) Internalization

It was demonstrated that protein-F1-mediated entry to cells and is involved in the causative process of the carriage state. As penicillin does not gain high intracellular concentrations, cell internalization was proposed as another possible explanation for penicillin treatment failures in children with pharyngitis.

By using a human epithelial cell line model, Kaplan et al.[9], showed that penicillin was less effective in killing intracellular streptococci than cephalothin, clindamycin, erythromycin or azithromycin.

(e) Suppression of natural immune response

It has been proposed that the success of penicillin treatment could be influenced by the delay of at least 2 days the beginning of antibiotic administration. However, there are several studies that do not agree with this theory[27].

(f) Biofilm formation

The role of biofilm formation in evading antibiotic eradication of GAS in patients with pharyngitis was not clearly proved, although, after treatments with high concentrations of antibiotics, *S. pyogenes* only survived in biofilm[22].

(g) Other explanations

Some defects in the design or performance of studies may erroneously categorize the causes of the outcome of treatments. Data presented in the literature do not often provide sufficient information for distinguishing between true eradication failure and recolonization following successful eradication[27].

There are some studies that do not recognize between true failures and GAS carriers that suffer viral infections. Furthermore it was suggested that the majority of reported treatment failures were in fact due to inappropriate dosage of the antibiotic, the duration of therapy and/or poor patient compliance.

Is penicillin still the antibiotic of choice for treating GAS pharyngitis?

According with current guidelines, penicillin V is still the antibiotic of choice for treating GAS pharyngitis[28].

The following are some of the reasons to continue recommending penicillin v for the treatment of GAS pharyngitis:

(1) Its lower cost compared to alternative agents.
(2) GAS isolates with reduced susceptibility to penicillin have not been described yet.
(3) Penicillin is among the best-tolerated antibiotics in non-penicillin-allergic individuals.
(4) It shows a narrow spectrum, causing less selection pressure for multidrug-resistant bacteria.
(5) Penicillin is the only antibiotic with proven efficacy to prevent acute rheumatic fever.

Reduced susceptibility to β-lactam antibiotics in *S. agalactiae*

Penicillin remains the first choice to treat GBS infections although since 2008, strains with reduced susceptibility to this antimicrobial agent have been described in Japan, USA, UK and Canada[4,6,13,16].

The prevalence is high in Japan, which was reported to be 2.3% in the period 2005–2006. It increased to 14.7% between 2012 and 2013. The latter GBS with reduced susceptibility to penicillin (PRGBS) were also multidrug-resistant (MDR), being 71.1% and 95.6% resistant to erythromycin and levofloxacin respectively, and 68.9% resistant to both antibiotics[26]. Therefore, the spread of PRGBS with a tendency to MDR, has increased in Japan. Most PRGBS were recovered from respiratory samples from elderly patients and some others, reported in Swedish and Japanese studies, were recovered from invasive samples from neonates and adults, but with only 3 PRGBS isolates in total[19,25].

Because the number of PRGBS is scarce, data regarding sequence type (ST) is poor. Kimura et al. found that ST1 and a single *locus* variant ST458 are predominant in isolates recovered between 1998 and 2003 periods and differ from the results obtained in USA, where ST19 is the only sequence type described so far[6,12].

In Argentina, GBS continue to be susceptible to penicillin[1,24].

Mechanisms of β-lactam resistance in *S. agalactiae*

Penicillin resistance in gram-positive organisms is essentially due to the production of altered, low-affinity target enzymes, penicillin-binding proteins (PBPs) that catalyze the terminal stage of bacterial cell wall peptidoglycan synthesis. In PBPs, three conserved motifs, SXXK, SXN, and KT(S)G, commonly found in transpeptidase domains form the catalytic center; and alterations within or adjacent to these motifs are associated with their reduced affinity for β-lactams[7].

Specifically, in GBS with reduced susceptibility to penicillin the substitutions occur mainly in PBP2X in amino acids V405A and/or Q557E[6,13,20]. Moreover, an amino acid substitution in PBP1A confers high-level resistance to cephalosporins[14] and multiple amino acid substitutions were found in PBP2X and PBP2B. These mutations are related to their penicillin MIC levels. Recently ceftibuten-resistant but penicillin-susceptible GBS with amino acid substitutions in PBP2X were reported[21].

Taking into account all the different cases that have been found in the reports related to changes in PBPs, Kimura et al.[10] proposed a classification for PRGBS based on amino acid substitutions and suggested considering whether the isolate has changes in PBP2X and other PBPs and resistance to other β-lactams.

How to phenotypically detect isolates with reduced susceptibility to penicillin?

It is important to mention that the use of 10 IU penicillin disks, as recommended by the CLSI, is not effective to demonstrate the presence of PRGBS. Therefore, dilution methods should be performed for MIC determination. CLSI guides state that if the MIC is $\leq$0.12 μg/ml, the isolate is susceptible to penicillin[3]; on the other hand, the EUCAST establishes a cutoff value of >0.25 μg/ml for the resistant category (http://www.eucast.org/clinical_breakpoints/). The study of the resistance mechanism involved in reduced susceptibility has to be done by sequencing PBP coding genes to detect substitutions in PBPs. As we mentioned, other β-lactams could have MIC values in the range of non-susceptibility. In the effort to detect PRGBS isolates without MIC determination, Kimura et al. developed a three disk (3 DM) screening method, using ceftibuten (CBT), oxacillin (OXA) and ceftizoxime (ZOX)[15]. Cutoff values are: to OXA < 17 mm, CBT < 29 mm and ZOX < 20 mm. In brief, in order to be included in PRGBS according to these criteria, the inhibition zone of 2/3 disks have to be below the cutoff value. Thus, in the isolates tested by Kimura, the growth inhibition zones around the PEN disk were >24 mm (CLSI susceptibility criteria) but the MIC values were >0.12 μg/ml. With this method they obtained good specificity and sensitivity. The use of 3 DM is controversial. When Cooper et al.[4] performed this screening they found nearly 50% of false positive rates with too low specificity. The same was found by Arias et al. in Argentina.[1]

Therefore, there is evidence that the 3 DM method is not recommended or, at least, breakpoints would be modified.

Another issue is that the Vitek 2 compact system with AST-P456 cards (BioMérieux Clinical Diagnostic, Marcy l'Etoile, France) only detects half of the PRGBS isolates, suggesting that many of these isolates would be misclassified as susceptible to penicillin. The authors also suggested that operators should pay attention if the system gives a result of MIC = 0.12 μg/ml[11].

Conclusion

It is important to highlight that confirmation by reference methods is mandatory when reduced susceptibility to penicillin is suspected in this group of bacteria, and the mechanism involved should be studied.

Conflict of interest

The authors declare that they have no conflicts of interest.

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