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Original Article

Phenotypic and molecular characterization of resistance to macrolides, lincosamides and type B streptogramin of clinical isolates of *Staphylococcus* spp. of a university hospital in Recife, Pernambuco, Brazil

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ABSTRACT

Introduction: There is a mechanism of macrolide resistance in *Staphylococcus* spp. which also affects the lincosamides and type B streptogramins characterizing the so-called MLS_B resistance, whose expression can be constitutive ($cMLS_B$) or inducible ($iMLS_B$) and is encoded mainly by *ermA* and *ermC* genes. The $cMLS_B$ resistance is easily detected by susceptibility testing used in the laboratory routine, but $iMLS_B$ resistance is not. Therapy with clindamycin in cases of infection with isolated $iMLS_B$ resistance may fail.

Objective: To characterize the phenotypic (occurrence of $cMLS_B$ and $iMLS_B$ phenotypes) and molecular (occurrence of *ermA* and *ermC* genes) profiles of MLS_B resistance of clinical isolates of susceptible and methicillin-resistant *Staphylococcus aureus* and CNS (coagulase-negative *Staphylococcus*) from patients of a university hospital, in Pernambuco.

Methods: The antimicrobial susceptibility of 103 isolates was determined by the disk diffusion technique in Mueller–Hinton agar followed by oxacillin *screening*. The $iMLS_B$ phenotype was detected by D test. Isolates with $cMLS_B$ and $iMLS_B$ phenotypes were subjected to polymerase chain reaction (PCR) for the detection of *ermA* and *ermC* genes.

Results: The $cMLS_B$ and $iMLS_B$ phenotypes were respectively identified in 39 (37.9%) and five (4.9%) isolates. The $iMLS_B$ phenotype was found only in four (10.8%) methicillin-susceptible *S. aureus* and one (4.5%) methicillin-resistant *S. aureus*. In the 44 isolates subjected to PCR, four (9.1%) only *ermA* gene was detected, a lower frequency when compared to only *ermC* 17 (38.6%) gene and to one (2.3%) isolate presenting both genes.

Conclusion: In the *Staphylococcus* spp. analyzed, the *ermC* gene was found more often than the *ermA*, although the $iMLS_B$ phenotype had been less frequent than the $cMLS_B$. It was important to perform the D test for its detection to guide therapeutic approaches.

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Introduction

The increasing prevalence of methicillin resistance in *Staphylococcus* spp. is a growing problem. This has renewed interest regarding the use of macrolide, lincosamides, and type B streptogramin antimicrobials for the treatment of staphylococcal infections. Clindamycin, a lincosamide, represents a common choice for some of these infections, particularly for infections of the skin and soft tissues, and an alternative in case of intolerance to penicillin or methicillin resistance.[1–3] In *Staphylococcus* spp., one of the resistance mechanisms consists of ribosomal target modification, affecting macrolides, lincosamides, and type B streptogramins characterizes the so-called MLS_B resistance. Its expression can be constitutive ($cMLS_B$) or inducible ($iMLS_B$) and is encoded by *ermA* (erythromycin ribosome methylase) and *ermC* genes, which are the main determinants of resistance in staphylococci.[2,4,5]

It is important to know the type of MLS_B resistance for establishing adequate therapy, since *Staphylococcus* spp. with constitutive resistance present *in vitro* resistance to all macrolides, lincosamides, and type B streptogramins. In addition, *in vivo* therapy with clindamycin for *Staphylococcus* spp. infection with inducible resistance can select constitutive *erm* mutants, resulting in treatment failure.[3,4,6,7] It is noteworthy that $cMLS_B$ resistance is easily detected by susceptibility testing used in the laboratory routine, while $iMLS_B$ resistance is not. Using these detection methods, *Staphylococcus* spp. with inducible resistance has *in vitro* resistance to erythromycin and susceptibility to clindamycin.[5,6,8,9]

To detect the inducible clindamycin resistance in *Staphylococcus* spp., one of the tests recommended by the CLSI (Clinical and Laboratory Standards Institute) is the double-disk diffusion test (D Test) and when the isolated present such resistance, the CLSI recommends reporting them as resistant to clindamycin.[10] Then, data on the antimicrobial susceptibility are important in the choice of therapy against infections, but false susceptibility results can be obtained if the isolates are not subjected to tests that detect inducible resistance to clindamycin.[11]

Studies carried out in two Brazilian states with clinical isolates of *Staphylococcus* spp. reported the $cMLS_B$ phenotype as the most frequent.[12,13] Coutinho et al.[13] have also evaluated the occurrence of the *erm* genes among the isolates analyzed. However, the frequency of $cMLS_B$ and $iMLS_B$ resistance varies among different hospitals and there are other resistance mechanisms that confer resistance to only one or two classes of the MLS_B complex.[14,15]

The objective of this study was to characterize the phenotypic (occurrence of $cMLS_B$ and $iMLS_B$ phenotypes) and molecular (occurrence of *ermA* and *ermC* genes) profiles of MLS_B resistance of clinical isolates of susceptible and methicillin-resistant *Staphylococcus aureus* and CNS (coagulase-negative *Staphylococcus*) from patients of a university hospital in Pernambuco, Brazil. Obtaining local data relating to resistance, may be helpful in guiding therapeutic approaches.

Materials and methods

Clinical isolates

A total of 103 clinical isolates were gathered from various samples from patients infected with *S. aureus* or SCN of a university hospital of Pernambuco, Brazil, during the year 2012 and were stored in glycerol (25%) at −20 °C. To verify the purity, the colonies were inoculated into Brain Heart Infusion broth (BHI) and after incubation at 37 °C for 48 h were plated on blood agar.

Antimicrobial susceptibility profile

The antibiogram was performed by disk diffusion technique in Mueller–Hinton agar, using antibiotic clindamycin 2 μg, erythromycin 15 μg, cefoxitin 30 μg, and oxacillin 1 μg. The results were interpreted according to the standards determined by CLSI.[10]

Screening for oxacillin resistance

Isolates with resistance or intermediate resistance to oxacillin and/or cefoxitin were submitted to oxacillin *screening*, as proposed by Rabelo et al.[16]

D test

S. aureus and SCN isolates with resistance to erythromycin and susceptibility or intermediate resistance to clindamycin in the antibiogram were selected. For the execution of this test a disk of 2 μg of clindamycin was placed at a distance of 15 mm–26 mm from the edge of a disk of 15 μg of erythromycin in a plate containing Mueller–Hinton agar sown in the same way as it was for the antibiogram. After incubation at 35 °C for 16–18 h, isolates that showed no flattening of the inhibition zone around the clindamycin disk were reported as susceptible to clindamycin (negative D test) and isolates that showed flattening of the inhibition zone around the clindamycin disk adjacent to erythromycin disk ("D" zone) indicated inducible clindamycin resistance (positive D test).[10]

Extraction of total DNA

To examine the presence of *ermA* and *emrC* genes, the rapid extraction of the total DNA of isolates that showed phenotypes MLS_Bc and MLS_Bi was performed by a modified technique of thermal lysis directly from the colony, according to Hu et al.,[17] after inoculation of a colony of each isolate into 5 mL of BHI and incubated at 37 °C for 24 h.

Polymerase chain reaction (PCR) conditions

PCR was performed using the primers described by Lina et al.[18] for *ermA* and *ermC* genes. For the detection of *ermA* gene, each amplification reaction was prepared in a final volume of 25 μL for each tube and includes: 1 μL (40 ng) of total DNA, 1 μL (20 pmol) of each primer, 0.6 μL of deoxyribonucleotide triphosphate (dNTP) (8 mM), 5.0 μL of buffer (5×), 1.5 μL of