Clindamycin microbial resistance in clinical isolates of *Staphylococcus* sp. derived from blood cultures of hospitalized patients

Resistência microbiana à clindamicina em isolados clínicos de Staphylococcus sp. provenientes de hemoculturas de pacientes hospitalizados

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ABSTRACT

Introduction: Infections caused by methicillin-resistant Staphylococcus aureus (MRSA) strains have become common in hospitals, and this resistance has limited patients' treatment with beta-lactam antibiotics. Clindamycin is an important therapeutic agent for MRSA infections; however, inducible macrolide-lincosamide-streptogramin B (MLS_B) resistance (iMLS_B) has resulted in therapeutic failures with the use of this antimicrobial, with a consequent increase in patient mortality and length of stay. **Objective**: This study was carried out in order to detect phenotypes of inducible and constitutive resistance to clindamycin in blood culture samples of Staphylococcus sp. from hospitalized patients. **Material and methods**: A hundred bacterial samples from blood cultures of adult patients were evaluated, identified by conventional phenotypic tests, and subject to determination of susceptibility and resistance profiles with cefoxitin, erythromycin, and clindamycin discs; and to phenotypic evaluation of iMLS_B resistance using the D-test. The results were interpreted in accordance with the recommendations of the Clinical and Laboratory Standards Institute (CLSI) 2014. **Results**: In this study, 65% of 60 bacterial strains were susceptible to cefoxitin and 35%, resistant. The constitutive MLS_B (cMLS_B) resistance phenotype was observed in 15.56% of the methicillin-sensitive Staphylococcus aureus (MSCONS), 26.67% of MRSA and 20% of methicillin-resistant coagulase-negative Staphylococcus (MRCONS), while iMLS_B resistance was observed only in strains susceptible to cefoxitin, corresponding to 80% in MSSA and 20% in MSCONS. **Conclusion**: The D-test is a key test for the constant monitoring of the inducible resistance phenotype (which may impair the effectiveness of treatment with clindamycin), minimizing potential medication errors and adverse clinical outcomes.

Key words: methicillin-resistant Staphylococcus aureus; clindamycin; microbial sensitivity tests.

INTRODUCTION

Although a member of the human microbiota, *Staphylococcus* sp. is a major pathogen responsible for a variety of diseases ranging in severity from skin and soft tissue infections to life-threatening conditions such as endocarditis, pneumonia and sepsis⁽¹⁾. Its clinical importance has grown, in particular as a result of the increase in serious hospital infections caused by strains of methicillin-resistant *Staphylococcus aureus* (MRSA)⁽²⁾. The microorganism stands out especially for its high frequency and pathogenicity that enables it to produce disease in both

immunocompromised and healthy individuals, and its easy inhospital spread associated with antimicrobial resistance $^{(3,4)}$.

MRSA strains are considered resistant to all beta-lactams (penicillins, cephalosporins, carbapenems and monobactams), regardless of antibiogram results $^{(3)}$. These usually lead to a constant interest in the use of macrolides (erythromycin, azithromycin, clarithromycin), lincosamides (clindamycin) and streptogramin B (quinupristin-dalfopristin), which correspond to the macrolide-lincosamide-streptogramin B (MLS_R) group.

Clindamycin is the preferred antibiotic for the treatment of MRSA strains due to its excellent pharmacokinetic properties, such

as optimum tissue penetration and abscess accumulation⁽¹⁾. It acts in connection with the 23S ribosomal ribonucleic acid (RNA) of the 50S subunit, interfering with the elongation of the peptide chain and blocking the synthesis of bacterial proteins⁽⁵⁾. However, the misuse of MSL_B antibiotics has led to increased numbers of *Staphylococcus* sp. resistant to these drugs due to a variety of resistance mechanisms, including, decreased permeability, alteration of the site of action in the ribosome, enzymatic action and efflux pumps⁽⁶⁾.

Depending on the exposure, MLS_B resistance may be constitutive (cMLS_B) or inducible (iMLS_B). In cMLS_B expression, microorganisms have the erythromycin ribosomal methylase (*erm*) gene and are resistant to erythromycin and clindamycin; while in iMLS_B the *erm* gene requires an inducing agent, rendering the messenger RNA (mRNA) active and producing methylases; resistance to erythromycin and false sensitivity to clindamycin *in vitro* (2,7) are observed. Besides these phenotypes, resistance can still be associated with expression of the *mef*A gene, whose samples show M phenotype (resistance only to macrolides), or expression of the *lin*B gene, with resistance only to clindamycin (8).

The in vitro susceptibility testing using the standard disk-diffusion method with erythromycin and clindamycin may not detect the iMLS_B phenotype. The Clinical and Laboratory Standards Institute (CLSI) recommended detecting this resistance by the D-test, a phenotypic method that consists of placing the erythromycin disk at a distance of 20 mm from the clindamycin disk⁽⁹⁾. The presence of a "D zone" should be interpreted as clindamycin resistance, regardless of the diameter of the found inhibition halo. If this positive result is ignored, treatment with clindamycin will be subject to therapeutic failures⁽¹⁰⁾.

Given the high transmissibility of resistance genes between strains, and the abusive use of antimicrobials, by selecting multiresistant samples this study aims to determine the prevalence of constitutive and inducible clindamycin resistance in blood culture samples of *Staphylococcus* sp. from patients admitted to Hospital do Agreste Pernambucano, located in the city of Caruaru, Pernambuco, Brazil.

MATERIAL AND METHODS

Place of study and its population

Between August and December 2014, we analyzed 100 blood culture samples from patients admitted to Hospital do Agreste Pernambucano, located in the city of Caruaru, Pernambuco, Brazil.

Collection and processing of samples

From the blood culture bottles provided by the hospital's microbiology laboratory, aliquots were aspirated for preparation of slides, which were stained with the Gram method and sown in sheep blood agar and MacConkey agar. From this primary inoculation, a presumptive identification was made, given that the Gram-positive strains only grew amid sheep blood agar. Based on the colonies obtained in this medium, the phenotypic identification was carried out at gender level and also, when possible, species level, according to the macro and microscopic characteristics of the colonies and the results of the catalase, deoxyribonuclease (DNase) and novobiocin tests.

Determination of pattern of sensitivity and resistance

The identified Gram-positive strains underwent disk-diffusion test to be classified as susceptible, intermediate or resistant to cefoxitin (30 µg CFO), clindamycin (2 µg) and erythromycin (15 µg) antibiotics. In order to perform the test, bacterial suspension adjusted to 0.5 McFarland standard inoculated the surface of Mueller-Hinton agar plates. Subsequently, the disks were placed, and the plates were incubated in a bacteriological oven at 35°C-37°C for 18-24 hours. The diameters of the inhibition zones were interpreted, following the guidelines of the CLSI 2014, as follows: sensitive (S) \geq 22 mm, and resistant (R) \leq 21 mm to cefoxitin, in the case of *Staphylococcus aureus*; S \geq 25 mm and R \leq 24 mm to cefoxitin, in the case of coagulase-negative *Staphylococcus* and *Staphylococcus saprophyticus*; S \geq 23 mm, intermediate (I) = 14-22 mm and R \leq 13 mm to erythromycin; and S \geq 21 mm, I = 15-20 mm and R \leq 14 mm to clindamycin (9).

Determination of inducible and constitutive MLS_B resistance

The isolates with inhibition zones ≤ 13 mm for erythromycin and ≤ 14 mm for clindamycin have been reported as resistant, indicating cMLS_o resistance.

The erythromycin-resistant clindamycin-sensitive strains underwent the D-test for detection of inducible clindamycin resistance. To perform the test, the erythromycin disk was placed on the surface of a Mueller-Hinton agar plate previously inoculated with the bacterial suspension at a distance of 20 mm (edge to edge) of the clindamycin disk. The erythromycin present in the disk has spread from the culture medium and induced resistance to clindamycin, resulting in a flattening of the inhibition zone adjacent to the

erythromycin disk, in the shape of the letter D (D effect or zone). Thus, when the inhibition zone of the erythromycin disk is ≤ 13 mm and the clindamycin disk is ≥ 21 mm, and both portions are circular, the test is considered negative for iMLS_o resistance⁽²⁾.

Ethical considerations

This study was approved by the research ethics committee of Associação Caruaruense de Ensino Superior e Técnico (Asces), under report 421.338.

RESULTS

Out of the 100 samples analyzed from blood cultures, 60 were identified as Gram-positive bacteria that were classified according to the phenotypic identification under three different species: 48.33% (29 strains) *Staphylococcus aureus*, 45% (27 strains) coagulase-negative *Staphylococcus*, and 6.67% (four strains) *Staphylococcus saprophyticus* (**Figure 1**).

Among the 60 examined samples, 65% (39) were susceptible to cefoxitin, 43.59% (17) were phenotypically identified as methicillin-sensitive *Staphylococcus aureus* (MSSA), 51.29% (20) as methicillin-sensitive coagulase-negative *Staphylococcus* (MSCONS), and 5.12% (two) as methicillin-sensitive *Staphylococcus saprophyticus* (MSSS); 35% (21) were resistant to cefoxitin, and 57.15% (12) were identified as MRSA, 33.33% (seven) as methicillin- resistant coagulase-negative *Staphylococcus* (MRCONS) and 9.52% (two) as methicillin-resistant *Staphylococcus saprophyticus* (MRSS) (**Figure 2**).

The cMLS $_{\rm B}$ phenotype was observed in 75% (45) of the isolates, iMLS $_{\rm B}$ resistance in 8.33% (five) (80% being identified in *Staphylococcus aureus* and 20% in coagulase-negative *Staphylococcus*), M phenotype in 1.67% (one MSSA strain), and the phenotypic expression of the *Lin*B gene in 1.67% (one MSSA strain). Concomitant sensitivity to erythromycin and clindamycin was observed in 10% (six) of the isolated samples, and this phenotype was present in MSCONS samples (corresponding to 50% [three]), in MSSA (33.33% [two]), and in MSSS (16.67% [one]).

The cMLS_B resistance was detected in 26.67% (12) of MRSA, 20% (nine) of MRCONS and 2.22% (one) MRSS; iMLS_B resistance was not observed in methicillin-resistant strains. In methicillin-susceptible strains, the cMLS_B phenotype was 15.56% (seven) for MSSA, 33.33% (15) for MSCONS and 2.22% (one) for MSSS; the iMLS_B phenotype was 80% (four) for MSSA isolates and 20% (one) for MSCONS (**Table**).

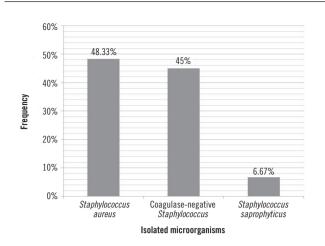


FIGURE 1 – Frequency of microorganisms isolated from the analyzed blood cultures

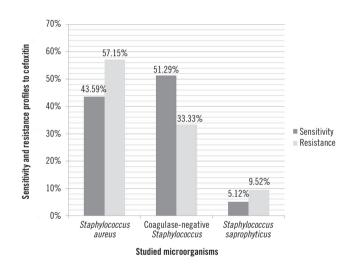


FIGURE 2 - Sensitivity and resistance profiles of Gram-positive strains to cefoxitin

TABLE – MLS_B resistance phenotypes of 60 strains of Staphylococcus sp. isolated from blood cultures

Resistance to cefoxitin	N (%)	M phenotype	<i>Lin</i> B gene expression	$\mathrm{iMLS}_{_{\mathrm{B}}}$	cMLS _B
MRSA	12 (57.15%)	-	-	-	12 (26.67%)
MSSA	17 (43.59%)	1 (1.67%)	1 (1.67%)	4 (80%)	7 (15.56%)
MRCONS	7 (33.33%)	-	-	-	9 (20%)
MSCONS	20 (51.29%)	-	-	1 (20%)	15 (33.33%)
MRSS	2 (9.52%)	-	-	-	1 (2.22%)
MSSS	2 (5.12%)	-	-	-	1 (2.22%)

 $MLS_{g'}$ macrolide-lincosamide-streptogramin B; $iMLS_{g'}$ inducible MLS_{g} phenotype; $cMLS_{g'}$ constitutive MLS_{g} phenotype; MRSA: methicillin-resistant Staphylococcus aureus; MSCA: methicillin-sensitive Staphylococcus; MSCONS: methicillin-sensitive coagulase-negative Staphylococcus; MSCONS: methicillin-sensitive coagulase-negative Staphylococcus; MSCONS: methicillin-resistant Staphylococcus saprophyticus; MSSS: methicillin-sensitive Staphylococcus saprophyticus.

DISCUSSION

Among the 60 samples of Gram-positive bacteria in the study, 48.33% were *Staphylococcus aureus* and 45%, coagulase-negative *Staphylococcus*. Similar results were obtained in a study conducted in São Paulo, by Alves *et al.* (2012), in which coagulase-negative *Staphylococcus* was prevalent (45.5%). This can be a result of the use of intravenous devices such as catheters — important sources of contamination because they are easily colonized by skin microorganisms⁽¹¹⁾.

Revealing a prevalence contrasting with the results of this study, Sousa *et al.* (2014) analyzed 89 Gram-positive samples of blood cultures from intensive care unit (ICU) patients: coagulase-negative *Staphylococcus* was the most prevalent microorganism (23.5%), followed by *Staphylococcus aureus* (21.15%), *Enterococcus* sp. (5.29%), *Streptococcus* sp. (1.76%) and *Leuconostoc* sp. (0.6%), bacterial genders not isolated in this study⁽¹²⁾.

Four strains of *Staphylococcus saprophyticus* were also identified. This microorganism is found in the rectum and more often in the female genital tract, being a usual agent of urinary tract infection (UTI). When it is present in blood cultures, contamination is generally attributed to the time of collection, but when bacteremia is significant, it may occur in patients with hematological malignancies and is predominantly associated with the use of the catheter. It can also be associated with the UTI after extracorporeal lithotripsy^(13, 14).

In general, rates of sensitivity and resistance to cefoxitin were 65% and 35%, respectively. The frequency of resistance was lower than that reported by Almeida *et al.* (2012) (75.1%), but much like the one found by Amorim *et al.* (2009) (29.25%)^(2,15). In this study, sensitivity to cefoxitin was observed, with a higher prevalence, in isolated coagulase-negative *Staphylococcus* (51.29%); however, resistance to cefoxitin was identified in a higher frequency in strains of *Staphylococcus aureus* (57.15%).

In the work by Junior *et al.* (2009), coagulase-negative *Staphylococcus* was found to have higher prevalence of cefoxitin-resistant strains than *Staphylococcus aureus* (71.1% vs. 18.1%), what is not consistent with our results, which showed that resistance is more pronounced in *Staphylococcus aureus* (33.33% vs. 57.15%)⁽¹⁶⁾. It is worth noting that the importance of this resistance is not just about frequency, but also the potential severity of infections and their form of dissemination. This demonstrates the need for prophylactic measures against the indiscriminate use of antibiotics, which brings as its main clinical consequence, the prolonged stay of infected patients in the hospital.

Methicillin-resistant *Staphylococcus* sp. is a major cause of bacteremia in ICU, restricting prophylaxis with cephalosporins due to intrinsic resistance by beta-lactamase. This condition contributes to increased morbidity and mortality, acquisition of secondary nosocomial infections and unsuccessful treatments, as a result of the few therapeutic options^(17, 18).

The iMLS_B phenotype was found only in cefoxitin-susceptible isolates, identified in four MSSA strains (80%) and one MSCONS (20%). Our results confirm the findings of studies by Ciraj *et al.* (2009) and Eksi *et al.* (2011), who found significant frequency of this phenotype in cefoxitin-susceptible strains^(19, 20).

Conversely, the study by Bottega *et al.* (2014), at a University Hospital in Santa Maria, Rio Grande do Sul, Brazil, indicated a higher prevalence of the $iMLS_B$ phenotype in MRSA strains, corresponding to 10.3%, compared to 7.2% prevalence of this phenotype in MSSA strains⁽¹⁾.

In this study, there was no statistically significant difference in the prevalence of $cMLS_B$ phenotype in MRSA strains (26.67%) compared to MSSA strains (20%). However, there was a higher prevalence of the phenotype in MSCONS isolates (33.33%) contrasted with MRCONS isolates (15.56%). These data confront the results found by Amorim *et al.* (2009), who observed the most common constitutive expression among cefoxitin-resistant isolates.

CONCLUSION

Although clindamycin is an option for treatment of infections caused by MRSA, the $\mathrm{iMLS}_{\mathrm{B}}$ phenotype may limit the effectiveness of this drug. Therefore, the D-test is essential for monitoring sensitivity to clindamycin in antimicrobial susceptibility *in vitro* tests in the routine of clinical laboratories, in an effort to minimize medication errors and ineffective treatments.

The frequency and evolution of infection by resistant *Staphylococcus* spp. in hospitalized individuals, especially in ICUs, demonstrates the need for immediate prophylactic measures in order to prevent the spread of this phenomenon. It is recommended that studies like this continue to be carried out to monitor infection control programs and resistance indices.

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RESUMO

Introdução: As infecções causadas por cepas de Staphylococcus aureus resistente à meticilina (MRSA) tornaram-se comuns no âmbito hospitalar, e essa resistência limitou o tratamento dos pacientes com antibióticos betalactâmicos. A clindamicina é um importante agente terapêutico para as infecções por MRSA, porém a resistência a macrolídeos, lincosamida e estreptogramina B (MLS_a) induzível (iMLS_a) tem conferido falhas terapêuticas ao uso desse antimicrobiano, com consequente aumento da mortalidade e do tempo de internação dos pacientes. Objetivo: Este estudo foi realizado com o propósito de detectar fenótipos de resistência constitutiva e induzível à clindamicina em amostras de hemoculturas do gênero Staphylococcus sp. de pacientes internados. Material e métodos: Foram analisadas 100 amostras bacterianas provenientes de hemoculturas de pacientes adultos, as quais foram identificadas por testes fenotípicos convencionais e submetidas à determinação do perfil de sensibilidade e resistência com discos de cefoxitina, eritromicina e clindamicina e à pesquisa fenotípica de resistência iMLS, pelo D-teste. Os resultados foram interpretados de acordo com as recomendações do Clinical and Laboratory Standards Institute (CLSI) 2014. Resultados: Neste estudo, 65% das 60 amostras bacterianas foram sensíveis à cefoxitina e 35%, resistentes. O fenótipo de resistência MLS, constituído (cMLS_a) foi observado em 15,56% das amostras de methicillin-sensitive Staphylococcus aureus (MSSA), 33,33% de methicillin-sensitive coagulase-negative Staphylococcus (MSCONS), 26,67% de MRSA e 20% de methicillin-resistant coagulase-negative Staphylococcus (MRCONS), enquanto a resistência iMLS, foi verificada apenas em isolados sensíveis à cefoxitina, correspondendo a 80% em MSSA e 20% em MSCONS. Conclusão: O D-teste é um teste essencial para o monitoramento constante do iMLS, (que pode comprometer a eficácia do tratamento com clindamicina), minimizando possíveis erros terapêuticos e desfechos clínicos desfavoráveis.

Unitermos: Staphylococcus aureus resistente à meticilina; clindamicina; testes de sensibilidade microbiana.

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