Revista da Sociedade Brasileira de Medicina Tropical 47(5):589-592, Sep-Oct, 2014
http://dx.doi.org/10.1590/0037-8682-0140-2014

Major Article

Evaluation of constitutive and inducible resistance to clindamycin in clinical samples of *Staphylococcus aureus* from a tertiary hospital

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ABSTRACT

Introduction: Infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA) have become common in hospitals and the community environment, and this wide resistance has limited patient treatment. Clindamycin (CL) represents an important alternative therapy for infections caused by *S. aureus*. Antimicrobial susceptibility testing using standard methods may not detect inducible CL resistance. This study was performed to detect the phenotypes of resistance to macrolides-lincosamides-streptogramin B (MLS_B) antibiotics, including CL, in clinical samples of *S. aureus* from patients at a tertiary hospital in Santa Maria, State of Rio Grande do Sul, Brazil. **Methods:** One hundred and forty clinical isolates were submitted to the disk diffusion induction test (D-test) with an erythromycin (ER) disk positioned at a distance of 20mm from a CL disk. The results were interpreted according to the recommendations of the Clinical and Laboratory Standards Institute (CLSI). **Results:** In this study, 29 (20.7%) of the 140 *S. aureus* samples were resistant to methicillin (MRSA), and 111 (79.3%) were susceptible to methicillin (MSSA). The constitutive resistance phenotype ($cMLS_B$) was observed in 20 (14.3%) MRSA samples and in 5 (3.6%) MSSA samples, whereas the inducible resistance phenotype ($iMLS_B$) was observed in 3 (2.1%) MRSA samples and in 8 (5.8%) MSSA samples. **Conclusions:** The D-test is essential for detecting the $iMLS_B$ phenotype because the early identification of this phenotype allows clinicians to choose an appropriate treatment for patients. Furthermore, this test is simple, easy to perform and inexpensive.

Keywords: Methicillin-resistant *Staphylococcus aureus*. Methicillin-susceptible *Staphylococcus aureus*. D-test. Clindamycin bacterial resistance.

INTRODUCTION

Staphylococcus aureus is responsible for a variety of diseases that range in severity from skin and soft tissue infections to life-threatening conditions, such as endocarditis, pneumonia and sepsis[1]. The clinical importance of *S. aureus* has grown particularly because of the increased occurrence of serious infections caused by methicillin-resistant *S. aureus* (MRSA)[2], which are among the most frequent bacteria in healthcare-associated infections (HAIs)[3].

Changes in susceptibility patterns to β-lactam antibiotics have led to a renewed interest in the use of macrolides-lincosamides-streptogramin B (MLS_B)[4] antibiotics. Clindamycin (CL) is the preferred agent for the treatment of MRSA due to its excellent pharmacokinetic properties, such as optimal tissue penetration and accumulation in abscesses[5,6]. Furthermore, CL is a frequent choice for treating staphylococcal infections because this antibiotic can be orally administered and is well tolerated[7,8]. However, the indiscriminate use of MLS_B antibiotics has led to an increase in the number of *Staphylococcus* spp. isolates that are resistant to these drugs[9].

MLS_B antimicrobials are structurally unrelated; however, these drugs are microbiologically related because of their similar modes of action. These drugs inhibit bacterial protein synthesis in susceptible organisms by reversibly binding to the 23S ribosomal ribonucleic acid (rRNA) receptor of the 50S ribosomal subunit[10].

The MLS_B resistance phenotype can be either constitutive [constitutive resistance to CL ($cMLS_B$)] or inducible [inducible resistance to CL ($iMLS_B$)]. Organisms that express erythromycin ribosomal methylase (*erm)* genes may exhibit *in vitro* resistance to erythromycin (ER), CL and other drugs of the MLS_B group. This resistance is referred to as the $cMLS_B$ phenotype. However, organisms with *erm* genes that requires an inducing agent to express CL resistance, have the $iMLS_B$ phenotype, which is resistant to ER and falsely susceptible to CL *in vitro*[11].

Antimicrobial susceptibility testing using standard methods that involve broth or agar dilutions erythromycin/azithromycin disk diffusion that is not adjacent to CL, may

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Received 11 June 2014
Accepted 21 October 2014

not detect the $iMLS_B$ phenotype[8]. The $iMLS_B$ phenotype may limit the effectiveness of CL, thereby increasing the chance of therapeutic failures[12]. The Clinical and Laboratory Standards Institute (CLSI) recommends performing the disk diffusion induction test (D-test), which is a phenotypic screening method for inducible CL resistance[13].

This study aimed to determine the prevalence of constitutive and inducible CL resistance in clinical samples of *S. aureus* from patients at a tertiary hospital in Santa Maria, State of Rio Grande do Sul, Brazil.

METHODS

Staphylococcus aureus isolates were obtained from 140 different clinical specimens (e.g., urine, blood, and respiratory tract secretions) from patients who were treated at the University Hospital of Santa Maria (HUSM), from April 2011 to December 2011. The Department of Microbiology at the Clinical Analyses Laboratory identified the samples as *S. aureus*using phenotypic (Gram staining, catalase and coagulase tests and D-test) and automated (MicroScan®, Siemens Healthcare Diagnostics, Deerfield, IL, USA) methods.

The strains were stored in tryptone soya broth, which contained glycerol 15%, at -80°C. The strains were kept in the Bacterial Collection of the Bacteriology Laboratory of the Department of Clinical and Toxicological Analyses at the Federal University of Santa Maria (UFSM).

All of the collected isolates were submitted to antimicrobial susceptibility testing using the D-test (Kirby-Bauer) to classify the strains as susceptible, intermediate or resistant to cefoxitin (CFO, 30µg) (Sensidisc®, BD Diagnostics, New Jersey, USA); CL (2µg) (Sensidisc®, BD Diagnostics, New Jersey, USA) and ER (15µg) (Sensifar®, Cefar Diagnóstica Ltda, São Paulo, SP, Brasil). *S. aureus* ATCC 25923 was used as the quality control strain for the discs, as recommended by the CLSI[13].

The strains were inoculated in tryptone soya agar and incubated for 24h in a bacterial incubator (35ºC ± 2ºC). The bacterial inoculum was prepared in a sterile solution, and the turbidity was adjusted to McFarland standard 0.5. The suspensions were inoculated in Mueller-Hinton agar and incubated under the previously described conditions. Oxacillin resistance was detected in the MRSA strains using the CFO disc-diffusion method and the oxacillin broth microdilution method[13] with the MicroScan® automated system (Siemens Healthcare Diagnostics, Deerfield, IL, USA).

Strains that were resistant to ER and susceptible to CL were submitted to the D-test to detect inducible CL resistance. The ER disk was placed at a distance of 20mm (center to center) from the CL disk and incubated for a period of 18h[13]. The inhibition zone diameters were interpreted as follows: susceptible (S) ≥ 23mm; intermediate (I) = 14 to 22mm; resistant (R) ≤ 13mm for ER; S ≥ 21mm; I = 15-20mm; R ≤ 14mm for CL; and S ≥ 22mm and R ≤ 21mm for CFO. When the zone diameter of the ER disk was ≤ 13mm, and the diameter of the CL disk was ≥ 21mm and when both zones were circular, the test was considered to be negative for inducible resistance (negative D-test). When the zone diameter of ER disk was ≤ 13mm and the diameter of the CL disk was ≥ 21mm and the inhibition zone around the CL disc was D-shaped, the test was considered to be positive for inducible resistance (positive D-test).

Ethical considerations

This study was approved by the Research Ethics Committee (CEP) of the UFSM under approval number 0117.0.243.000-08.

RESULTS

Of the 140 samples of *S. aureus*, 29 (20.7%) were identified as MRSA and 111 (79.3%) as methicillin-sensitive *Staphylococcus aureus* (MSSA). The $cMLS_B$ phenotype was observed in 20 (14.3%) MRSA samples, and the $iMLS_B$ phenotype was observed in 3 (2.1%) MSSA samples. Additionally, the $cMLS_B$ phenotype was found in 5 (3.6%) MSSA samples, whereas the $iMLS_B$ phenotype was found in 8 (5.8%) MSSA samples.

Concomitant susceptibility to CL and ER was observed in most (57.9%) of the isolate samples, and this phenotype was predominant in the MSSA samples. Other susceptibility profiles were found in 21 isolates as shown in **Table 1**. The clinical samples with *S. aureus* isolates are described in **Table 2**.

DISCUSSION

Of the 140 samples of *S. aureus* in this study, 20.7% were MRSA and 79.3% were MSSA. Similar results were obtained in a study conducted in India by Ciraj et al.[10] where in the MRSA prevalence was 17.3%. The relatively low frequency of MRSA in the present study may be due to the work of the Infection Control Hospital Committee, which has been developing measures to control the use of antibiotics and to implement proper hygienization practices by the hospital staff of HUSM since 2006.

The $iMLS_B$ phenotype was found in 3 (10.3%) of the 29 MRSA samples and in 8 (7.2%) of the 111 MSSA samples. Our results confirm the findings in the study by Juyal et al.[11] in which a higher frequency of the $iMLS_B$ phenotype was found in MRSA strains (19.4%) than in MSSA strains (6.3%).

However, the study by Amorim et al.[14] at Marília Medical School Hospitals in State of São Paulo, Brazil and the study by Eksi et al.[15] at Gaziantep University Hospital in Turkey indicated a higher prevalence of the $iMLS_B$ phenotype in MSSA strains. The incidence of this phenotype in *S. aureus* varies according to the population of patients studied, geographic region, hospital characteristics and susceptibility or resistance to methicillin[8,16].

In the present study, we found a higher prevalence of the $cMLS_B$ phenotype in the MRSA strains (20/29; 68.9%) compared with the MSSA strains (5/111; 4.5%). In addition, other authors have found a higher frequency of constitutive resistance in MRSA isolates. Prabhu et al.[17] observed the $cMLS_B$ phenotype in 16.7% of the MRSA strains, and Seif et al.[18] observed this phenotype in 52.3% of MRSA strains.

TABLE 1 - The erythromycin and clindamycin susceptibility profiles of clinical samples of *Staphylococcus aureus.*

	MRSA		MSSA		Total	
Phenotype	n	%	n	%	n	%
ER–S, CL–S	4	2.9	77	55.0	81	57.9
ER–R, CL–R (constitutive MLS_B)	20	14.3	5	3.6	25	17.9
ER–R, CL–S (D-test +) (inducible MLS_B)	3	2.1	8	5.8	11	7.9
ER–R, CL–S (D-test -)	1	0.7	1	0.7	2	1.4
ER–S, CL–R	0	0.0	2	1.4	2	1.4
ER–S, CL–I	0	0.0	7	5.0	7	5.0
ER–I, CL–S	0	0.0	10	7.1	10	7.1
ER–I, CL–I	1	0.7	0	0.0	1	0.7
ER–I, CL–R	0	0.0	1	0.7	1	0.7
Total	29	20.7	111	79.3	140	100.0

MRSA: methicillin-resistant *Staphylococcus aureus*; **MSSA:** methicillin-sensitive *Staphylococcus aureus*; **ER:** erythromycin; **CL:** clindamycin; **S:** susceptible; **R:** resistant; **I:** intermediate; **MLS_B:** macrolides-lincosamides-streptogramin B.

TABLE2 - Isolation of *Staphylococcus aureus* in clinical samples.

Clinical samples	Total samples collected	Number of samples with iMLSB resistance
General secretions*	48	3
Respiratory tract secretions**	37	1
Peripheral blood	31	2
Urine	11	2
Body fluids***	7	2
Catheter's tip	4	0
Breast abscess and soft tissue	2	1
Total	140	11

$iMLS_B$: inducible resistance to clindamycin. *Secretion from surgical wounds, pressure ulcers and bone tissue. **Sputum, broncho-alveolar lavage and tracheal aspirates. ***Pleural, peritoneal, pre-patellar and dialysis.

Among the clinical samples of *S. aureus*, the $iMLS_B$ phenotype was identified more frequently in general secretions, peripheral blood, urine andbody fluids.

In this study, we found a higher frequency of the $cMLS_B$ and $iMLS_B$ phenotypes in MRSA strains. Therefore, the D-test is essential for monitoring susceptibility to CL and should be included in routine antimicrobial susceptibility testing because the inducible resistance phenotype can inhibit the action of CL, thereby rendering treatment ineffective. In addition, decisions regarding the method for routinely detecting *Staphylococcus* spp. with the $iMLS_B$ phenotype (ER-R/I, CL-S) should be discussed at individual institutions based on local data.

ACKNOWLEDGMENTS

We gratefully acknowledge the pharmaceutical staff of the Clinical Analyses Laboratory at HUSM.

CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

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