Available online at www.sciencedirect.com

ScienceDirect

journal homepage: www.elsevier.com/locate/mjafi

Original Article

Prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples

Lt Col Mahima Lall [a,*], *Brig A.K. Sahni* [b]

[a] *Classified Specialist (Pathology & Microbiology), Dept of Pathology & Molecular Medicine, Army Hospital (R&R), Delhi Cantt 110010, India*

[b] *Professor & HOD, Department of Microbiology, Armed Forces Medical College, Pune 40, India*

ARTICLE INFO

Article history:
Received 17 April 2012
Accepted 1 January 2013
Available online 8 May 2013

Keywords:
D test
Inducible clindamycin resistance
Staphylococcus aureus

ABSTRACT

Background: Therapy for Staphylococcal infections may be complicated by the possibility of inducible macrolide-lincosamide-streptogramin B resistance (MLSBi). We studied the prevalence of MLSBi in community associated (CA) and hospital associated (HA) *Staphylococcus aureus* isolates from clinical samples.

Methods: A total of 305 strains of *S. aureus* comprising 140 (45.9%) [95% CI 40.36–51.52] methicillin resistant *S. aureus* (MRSA) and 165 (54%) [95% CI 48.48–59.64] methicillin-sensitive *S. aureus* (MSSA) were identified by conventional methods. The double disc test (D test) was applied by placing erythromycin and clindamycin discs to investigate inducible and constitutive MLSBi resistant phenotypes.

Results: 16.6% of MRSA showed constitutive resistance and 37.5% inducible MLSBi resistance. Community associated MRSA (CA-MRSA) represented 10% of all isolates and had lower prevalence of MLSBi than hospital associated MRSA (HA-MRSA).

Conclusion: Routine screening for inducible MLSBi resistance by double disc test can screen for potential treatment failures such that clindamycin can be used effectively and judiciously when indicated for staphylococcal infections especially for treating skin and soft tissue infections (SSTIs) in CA-MRSA due to low prevalence of MLSBi among CA-MRSA.

Introduction

Staphylococcus aureus (*S. aureus*) is increasingly recognized as a cause of hospital associated (HA) and community associated (CA) infections. Macrolide, lincosamide and streptogramin B (MLSB) antibiotics are commonly used in treatment of staphylococcal infections. Widespread use of MLSB antibiotics has led to an increase in resistance to these antibiotics especially clindamycin, amongst staphylococcal strains.[1–3] Macrolides such as erythromycin, roxithromycin, clarithromycin and lincosamides such as clindamycin and lincomycin belong to different classes of antimicrobials but act through the same mechanism that is by inhibition of protein synthesis.[4] Clindamycin has long been an option for treating both methicillin-susceptible *S. aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) infections. Expression of inducible resistance to clindamycin could limit the effectiveness of this drug.[5] Macrolide

* *Corresponding author.*
E-mail address: drmahimalall@yahoo.com (M. Lall).

0377-1237/$ – see front matter © 2013, Armed Forces Medical Services (AFMS). All rights reserved.
http://dx.doi.org/10.1016/j.mjafi.2013.01.004

resistance may be constitutive or inducible in the presence of a macrolide inducer.[6]

This mechanism can be constitutive, where methylase is always produced, or can be inducible, where methylase is produced only in presence of a macrolide inducer. Among MLSB drugs only macrolides are good inducers of the enzyme erythromycin ribosome methylase (*erm*). Once induced, the gene product confers cross-resistance to other members of the group including lincosamides and streptogramin B.[7] *S. aureus* isolates with constitutive resistance show resistance to erythromycin and clindamycin on in vitro testing, whereas isolates with inducible resistance show resistance to erythromycin but appear sensitive to clindamycin on disc diffusion testing. A double disc diffusion test (D test) for detecting inducible resistance to clindamycin in erythromycin-resistant isolates can be performed by placing a 15 μg erythromycin disc in proximity to a 2 μg clindamycin disc in adjacent positions.[8] This test helps to distinguish staphylococci that have inducible resistance from those with constitutive resistance. For erythromycin-resistant isolates, D test can help to determine whether clindamycin could be used as a therapeutic option (reported as susceptible when the D test is negative or reported as resistant when the D test is positive). Data describing MLSBi prevalence or clinical predictors of the presence of macrolide-lincosamide-streptogramin B resistance (MLSBi) among community acquired methicillin resistant *S. aureus* (CA-MRSA) and hospital acquired methicillin resistant *S. aureus* (HA-MRSA) isolates are limited. In the present study, we aimed to determine the prevalence of MLSBi resistance in both hospital and community-associated *S. aureus* isolates, including MRSA and MSSA.

Materials and methods

This study included 305 nonduplicate isolates of *S. aureus* from various clinical samples. Isolated microorganisms were identified by using conventional methods (colony morphology, Gram stain, catalase test, slide and tube coagulase test and DNase test). Methicillin resistance was detected using 1 μg oxacillin disc (HiMedia) on a swab inoculated Mueller–Hinton agar plate supplemented with 2% NaCl and incubating at 35 °C for 24 h.

Antimicrobial susceptibilities were studied by Kirby–Bauer disc diffusion method as per guidelines from Clinical and Laboratory Standards Institute (CLSI). Interpretation of the diameters of zones of inhibition was as depicted in Table 1.

To detect inducible clindamycin resistance, 15 μg erythromycin and 2 μg clindamycin discs (HiMedia) were placed on Mueller–Hinton plate that had been inoculated with a staphylococcal isolate. The discs were placed at a distance of 15–20 mm edge to edge from each other. Plates were incubated overnight at 37 °C. *S. aureus* ATCC 25923 was used as control for these tests.

A positive D test was taken as flattening of the zone of inhibition around clindamycin disc proximal to erythromycin disc (D shaped zone of inhibition) and was defined as inducible MLSBi resistance (Fig. 1). Strains that were resistant to both erythromycin and clindamycin were defined as exhibiting constitutive MLSB resistance, and those that were resistant to erythromycin and sensitive to clindamycin were the MS phenotype.[9] The D test phenotype categories were recorded as noted in Table 2.

Medical records for the source patients were reviewed for presence of major comorbid conditions such as diabetes mellitus, post surgical status, malignancy, solid-organ transplant, trauma, and burn injury. Based on available records, determination was made as to whether a clinical infection due to the *S. aureus* cultured was present, as opposed to asymptomatic colonization. Infection was assumed to be present in all cases in which bacterial isolates were derived from blood or cerebrospinal fluid. MRSA isolates were designated HA-MRSA if the source patient had any of the following risk factors or comorbidities: a history of hospitalization, dialysis or surgery in recent past; growth of MRSA 48 h or more upon admission to a hospital; presence of a permanent indwelling catheter or percutaneous device at the time of culture; or prior positive MRSA culture. If none of the above risk factors was present, isolate was considered CA-MRSA.

Statistical analysis

Univariate analysis was carried out. Chi-square test was used for categorical variables and student's 't' test was carried out for quantitative variables.

Table 1 – Interpretation of erythromycin and clindamycin zone sizes in *S. aureus*.[a]

	Sensitive	Intermediate	Resistant
Erythromycin	≥23 mm	14–22 mm	≤13 mm
Clindamycin	≥21 mm	15–20 mm	≤14 mm

[a] CLSI Guidelines 2010: Performance std for Antimicrobial Disk Susceptibility Tests.

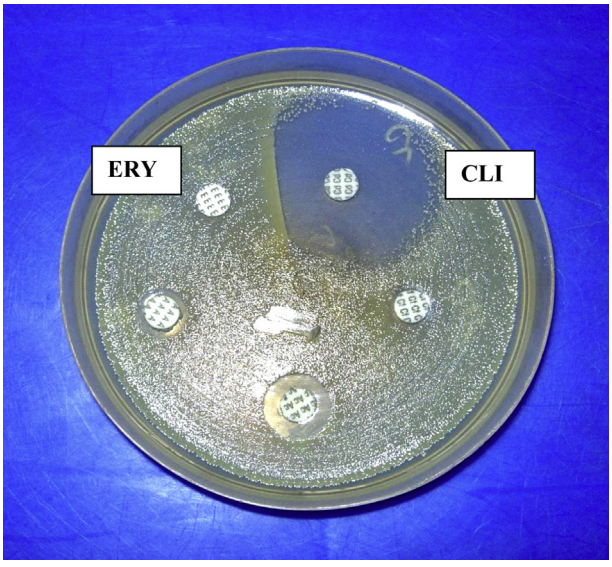

Fig. 1 – Double disc test (D test) showing flattening of the zone of inhibition around clindamycin disc proximal to erythromycin disc (D shaped zone of inhibition).