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 (MLS_{Bi}). We studied the prevalence of MLS_{Bi} 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 MLS_{Bi} resistant phenotypes.

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

Conclusion: Routine screening for inducible MLS_{Bi} 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 MLS_{Bi} among CA-MRSA.

© 2013, Armed Forces Medical Services (AFMS). All rights reserved.

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 (MLS_B) antibiotics are commonly used in treatment of staphylococcal infections. Widespread use of MLS_B 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.

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).

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.

Table 2 – D test phenotype categories and their characteristics.

D test phenotype	Resistance phenotype	CLI result	ERY result	Double disc test description
D+	Inducible MLSB	S	R	Blunted, D shaped clear zone around CLI disc proximal to ERY disc
D–	MS	S	R	Clear zone around CLI
R	Constitutive MLSB	R	R	Growth upto CLI and ERY discs
S	No resistance	S	S	Clear zone around discs

S – Sensitive, R – Resistant, CLI – Clindamycin, ERY – Erythromycin.

Results

During the study period, 305 *S. aureus* isolates were collected prospectively. Among these 140 (45.9%) [95% CI 40.36–51.52] were MRSA and 165 (54%) [95% CI 48.48–59.64] were MSSA. The difference in proportion was found to be statistically significant between the MRSA and MSSA ($p = 0.04$). The presence of MLSBi was confirmed by the D test. A blunted edge with otherwise clear zone of inhibition around clindamycin disc was seen in D test positive strains. The overall prevalence of MLSBi among all *Staphylococcus* isolates was 20.3% (Table 3).

In MRSA 37.5% exhibited the MLSBi, 16.6% exhibited the constitutive phenotype while 22.8% strains exhibited the MS phenotype. Among the 140 MRSA isolates, 30 (21.4%) were designated community associated MRSA (CA-MRSA) and 110 (78.5%) hospital associated MRSA (HA-MRSA). CA-MRSA represented 10% of all isolates.

The presence of MLSBi was detected in 23.3% CA-MRSA and 40.9% HA-MRSA. The proportion of MLSBi amongst HA-MRSA and CA-MRSA was compared and found to be statistically significant in HA-MRSA (86.5%).

Amongst MSSA, 6% exhibited the MLSBi, 8% the constitutive resistance phenotype while 31.5% exhibited MS phenotype. 93.94% exhibited sensitivity to clindamycin.

When the results were compared statistically for presence of inducible resistance in MRSA and MSSA, there was a highly significant difference amongst MRSA showing much higher proportion of MLSBi than MSSA (37.5%, 6% respectively) ($p = 0.000$). In our isolates the susceptibilities to erythromycin and clindamycin were higher in MSSA than among MRSA (93.94% versus 23.5% respectively). Univariate analysis revealed that methicillin resistance, classification into health care or community associated and presence of comorbidity were statistically significant factors ($p < 0.05$) as shown in Table 4.

Discussion

Empirical outpatient treatment options for staphylococcal infections have become more limited as concerns about the prevalence of MRSA have increased. Changing patterns in antimicrobial resistance have led to renewed interest in the use of clindamycin. Clindamycin is frequently used to treat skin and soft tissue infections (SSTIs) because of good oral bioavailability, low cost, excellent tissue penetration and the fact that it accumulates in abscesses. However therapeutic failures caused by MLSBi resistant strains are now being reported commonly.

Resistance to macrolide, lincosamide and streptogramin B (MLSB) antibiotics results from acquisition of *erm* gene. Expression of MLSB resistance can be constitutive or inducible.

Staphylococcal strains with MLSBi are resistant to inducer macrolides (eg. erythromycin) but susceptible to noninducer macrolides such as spiramycin and lincosamides (clindamycin) and streptogramin B (quinupristin). Inducible clindamycin resistance may not be detected if erythromycin and clindamycin discs are placed in nonadjacent positions.¹⁰ We found a high prevalence of 20.3% of MLSBi amongst all staphylococcal isolates. A study conducted in Turkey observed a prevalence of MLSBi as 21.9%.¹¹ Gadepalli et al found 21 per cent inducible MLSBi resistance phenotype.¹²

Our study suggests a higher prevalence of MLSBi in health care-associated than community associated *S. aureus* (86.5% versus 13.4% respectively). Implication of this finding is that patients who have infections due to CA-MRSA may reasonably be offered clindamycin as a treatment option because of a lower likelihood of the strains exhibiting MLSBi.

Different studies have found varying prevalence rates of inducible CLI resistance. In one study conducted at the university of Iowa, 65 (62%) of 105 *Staphylococcus* isolates showed MLSBi resistance phenotype.¹³ In another study at University

Table 3 – MLSB resistance phenotype of *S. aureus*.

	Total isolates	Constitutive MLSB resistance (ERY-R, CLI-R)	Inducible MLSB resistance (ERY-R, CLI-S, D+)	MS phenotype (ERY-R, CLI-S, D–)
<i>S. aureus</i>	305	–	–	–
MSSA	165 (54%)	8 (4.8%)	10 (6%)	52 (31.5%)
MRSA	140 (45.9%)	23 (16.6%)	52 (37.1%)	32 (22.8%)

ERY – Erythromycin, CLI – Clindamycin.

Table 4 – Comparison of D test positive and D test negative *S. aureus* isolates by the chi square analysis.

Variable	Isolates n = 305		MRSA isolate n = 140		Sex n = 305		Comorbidity n = 305	
	MRSA	MSSA	Healthcare associated	Community associated	Male	Female	Present	Absent
D+	52 (84%)	10 (16%)	45 (87%)	7 (13%)	44 (71%)	18 (29%)	47 (76%)	15 (24%)
D–	88 (36%)	155 (64%)	65 (74%)	23 (26%)	182 (75%)	61 (25%)	37 (15%)	206 (85%)
p Value	<0.001		0.077		0.528		<0.001	
Result	Highly significant		Not significant		Not significant		Highly significant	

of Texas Health Science Centre, 29% of 114 *S. aureus* isolates showed inducible resistance while 34% showed constitutive resistance to clindamycin.

In a study by Goyal et al, all MLSBi and MS phenotypes displayed resistance to macrolides and exhibited either a low level resistance or susceptibility to clindamycin before the induction by erythromycin. After induction with erythromycin, MICs of clindamycin were noticed to rise from 16 to 256 g/mL in MLSBi phenotypes indicating inducible resistance.¹⁴ Almost 50% of our isolates belonged to the MS Phenotype. These isolates showed resistance to erythromycin but were susceptible to clindamycin even in the presence of erythromycin, also 93.94% of MSSA isolates were susceptible to clindamycin suggesting a possible role of treatment with clindamycin in such strains.

Staphylococci exhibiting inducible resistance to MLS antibiotics are now common in clinical practice. Only a few reports describing patients who received clindamycin for *S. aureus* infections with MLSBi are available, and some of these patients developed constitutive resistance during therapy.^{3,15,16} One should be cautious about using clindamycin in patients with major infections, especially where treatment is likely to be prolonged or infection difficult to eradicate as constitutive mutants can be selected during the course of clindamycin therapy in patients with MLSBi.¹⁷

Currently, some authors recommend avoidance of clindamycin for treatment of complicated infections which may have a high bacterial burden, such as abscesses or osteomyelitis.¹⁸ Conversely labeling all erythromycin-resistant staphylococci as clindamycin resistant would prevent the use of clindamycin in infections caused by truly clindamycin susceptible isolates. Clindamycin may be useful for non-MLSBi infections esp. less severe *S. aureus* infections.

This study reflects the prevalence of MLSBi at a tertiary care centre; however prevalence may differ from institute to institute. Microbiological laboratories should adopt testing for MLSBi among *S. aureus* isolates and report isolates exhibiting MLSBi as clindamycin resistant.

Conflicts of interest

All authors have none to declare.

REFERENCES

1. Lim JA, Kwon AE, Kim SK, Chong Lee K, Choi EC. Prevalence of resistance to macrolide, lincosamide and streptogramin antibiotics in Gram-positive cocci isolated in Korean hospital. *J Antimicrob Chemother.* 2002;49:489–495.
2. Lina G, Quaglia A, Reverdy, Leclercq R, Vandenesch, Etienne. Distributon of genes encoding resistance to macrolides, lincosamides, and streptogramins among staphylococci. *Antimicrob Agents Chemother.* 1999;43:1062–1066.
3. Drinkovic D, Fuller ER, Shore KP, Holland DJ, Ellis Pegler. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. *J Antimicrob Chemother.* 2001;48:315–316.
4. Steward Christine D, Raney Patti M, Morell Allison, et al. Testing for induction of clindamycin resistance in erythromycin resistant isolates of *Staphylococcus aureus*. *J Clin Microbiol.* 2005;43:1716–1721.
5. Patel Mukesh, Waites Ken B, Moser Stephen A, Cloud Gretchen, Hoesley Craig. Prevalence of inducible clindamycin resistance among community and hospital associated *Staphylococcus aureus* isolates. *J Clin Microbiol.* 2006;44:2481–2484.
6. Delialioglu Nuran, Aslan Gonul, Ozturk Candan, Baki Vildan, Sen Sebahat. Inducible clindamycin resistance in staphylococci isolated from clinical samples. *J Infect Dis.* 2005;58:104–106.
7. Leclercq Roland. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis.* 2002;34:482–492.
8. Fiebelkorn KR, Crawford SA, McElmeel ML, Jorgensen JH. Practical disc diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. *J Clin Microbiol.* 2003;41:4740–4744.
9. Hamilton-Miller JMT, Shah. Patterns of phenotypic resistance to the macrolide-lincosamide-ketolide-streptogramin group of antibiotics in staphylococci. *J Antimicrob Chemother.* 2000;46:941–949.
10. Schreckenberger Paul, Ilendo Elizabeth, Ristow Kathryn. Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase – negative staphylococci in a community and a tertiary care hospital. *J.Clin Microbiol.* 2004;42:2777–2779.
11. Yilmaz Gurdal, Aydin Kemalettin. Detection and prevalence of inducible clindamycin resistance in staphylococci. *J Med Microbiol.* 2007;56:342–345.
12. Gadepalli Ravisekhar, Dhawan Benu, Mohanty Srujana, Kapil Arti. Inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus*. *Indian J Med Res.* 2006;123:571–573.
13. Sanchez, Flint, Jones. Occurrence of macrolide-lincosamide-streptogramin resistance among staphylococcal clinical isolates at a university medical center. Is false susceptibility to new macrolides and clindamycin a contemporary clinical and in vitro testing problem. *Diagn Microbiol Infect Dis.* 1993;16:205–213.
14. Goyal R, Singh NP, Manchanda V, Mathur M. Detection of clindamycin susceptibility in macrolide resistant phenotypes of *Staphylococcus aureus*. *Indian J Med Microbiol.* 2004;22:251–254.

15. Lewis JS, Jorgensen JH. Inducible clindamycin resistance in staphylococci: should clinicians and microbiologists be concerned? *Clin Infect Dis*. 2005;40:280–285.
16. Rao GG. Should clindamycin be used in treatment of patients with infections caused by erythromycin resistant staphylococci? *J Antimicrob Chemother*. 2000;45:715.
17. Levin TP, et al. Potential clindamycin resistance in clindamycin susceptible, erythromycin resistant *Staphylococcus aureus*: report a clinical failure. *Antimicrob Agents Chemother*. 2005;49:1222–1224.
18. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin resistant *Staphylococcus aureus* expressing inducible clindamycin resistance in vitro. *Clin Infect Dis*. 2003;37:1257–1260.

Journal Scan

Sui G.Y, Liu G.C, Liu G.Y, et al. Is sunlight exposure a risk factor for age related macular degeneration? A systematic review and meta-analysis. *Br J Ophthalmol*. 2013;97:389–394.

Age related macular degeneration (AMD) is a leading cause of blindness worldwide in the age group above 50 years. Two types have been described, the dry type which causes slow but irreversible blindness in advanced stages accounting for 90% of the total AMD and the wet type which causes rapid loss of vision and constitutes the remaining 10%. Presently the wet type is managed with intra-vitreous injection of anti-Vascular Endothelial Growth Factors (VEGF) whereas there is no curative treatment for advanced dry AMD. Current strategies are aimed at prevention of dry AMD. The risk factors that have been identified include age, gender, genetic factors, smoking, cardiovascular disease, and iris colour and sunlight exposure. However the data on sunlight exposure has been varied and rather controversial. In this article the authors have done a systematic review and meta-analysis to assess the association between sunlight exposure and AMD with the hypothesis that sunlight exposure is indeed a risk factor for AMD and that heterogeneity between studies is due to study-specific covariates, including latitude and gross domestic product (GDP) per capita. An electronic search was performed of PubMed, Web of Science and CNKI, which was supplemented by hand searching. The selection of studies, data abstraction and quality assessment were performed independently by three reviewers. Epidemiological studies that met both of the following items were selected: the studies concerning the association between AMD and sunlight exposure, and OR for the sunlight exposed/unexposed groups and their 95% CI were presented or the frequency of AMD in the exposed/unexposed group was available in the paper. Case reports, comments and experimental study designs in laboratory settings were

excluded. A random-effects meta-analysis, followed by subgroup analysis and sensitivity analysis, including a random-effects meta-regression for study-specific covariates were done. Fourteen studies were identified. Twelve studies identified an increasing risk of AMD with greater sunlight exposure, six of which reported significant risks. The pooled OR was 1.379 (95% CI 1.091–1.745). The subgroup of non-population-based studies revealed a significant risk (OR 2.018, 1.248–3.265, $p = 0.004$). The gross domestic product (GDP) per capita ($p = 0.048$), but not the latitude ($p = 0.21$), was identified as a factor that led to heterogeneity according to the meta-regression. The authors have concluded that the epidemiological literature published to date indicates that individuals with more sunlight exposure are at a significantly increased risk of AMD. The OR significantly decreased with increasing GDP per capita. Subgroup analysis suggested latitude might be a covariate that is negatively correlated with the strength of association, although the meta-regression offered insufficient evidence to support it. More studies using larger scales and better methodologies are needed to confirm these findings and identify other potential risk factors for AMD as well as clarify the uncertain relationship between OR strength and latitude.

Gp Capt M.A. Khan*

Associate Professor, Department of Ophthalmology,
Armed Forces Medical College, Pune-411040, India

Brig V.S. Gurunadh

Professor and Head, Department of Ophthalmology,
Armed Forces Medical Collage, Pune-411040, India

*Corresponding author.

E-mail address: mansurophthal@gmail.com (M.A. Khan)

0377-1237/\$ – see front matter
<http://dx.doi.org/10.1016/j.mjafi.2013.11.003>