

Short Communication

Prevalence of inducible clindamycin resistance among clinical isolates of *Staphylococcus aureus* detected by phenotypic method: A preliminary report

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Clindamycin is one of the alternative antibiotics in the therapy of *Staphylococcus aureus*, particularly in methicillin resistant *S. aureus* (MRSA) infections. But inducible clindamycin resistance (iMLSB) has been described as a cause of clinical failure of such infections. The present study attempted to evaluate the prevalence of inducible clindamycin resistance among *S. aureus* isolates in a tertiary care centre in north eastern India. The study was carried out in the department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow, India, during a period of one year from December 2008 to November 2009. It was a prospective cross sectional study. In total, 260 *S. aureus* isolates were subjected to routine antibiotic susceptibility testing, including cefoxitin (30 mg), by the Kirby Bauer disc diffusion method. Inducible resistance to clindamycin was tested by double disk diffusion assay (D test) as per Clinical and Laboratory Standards Institute (CLSI) guidelines. Overall, inducible clindamycin resistance was detected among sixty (23.2%) isolates, while 16(6.15%) showed constitutive resistance and the remaining 39 (15%) exhibited a MS phenotype. Inducible resistance and constitutive resistance were higher in MRSA than methicillin-susceptible *S. aureus* (MSSA). Therefore, owing to the high percentage of inducible clindamycin resistance, we recommend that a screening test such as the D test should be included in routine susceptibility testing for *S. aureus*.

Key words: Double disk diffusion assay (D test), inducible clindamycin resistance (iMLSB), *Staphylococcus aureus*.

INTRODUCTION

Resistance to antimicrobial agents among *Staphylococcus aureus* isolates has become an ever-increasing problem among hospitalized patients, persons in long-term care facilities and ambulatory outpatients. There are many options available for the treatment of methicillin sensitive (MSSA) and methicillin resistant (MRSA) staphylococcal infections, like clindamycin being one of the good alternatives, particularly for skin and soft tissue infections and as an alternative in penicillin allergic patients (Ajanta et al., 2008). Clindamycin, a lincosamide

is 100% bioavailable when given orally and so it is a convenient drug for outpatients or as a follow up drug after intravenous therapy. However, most *S. aureus* which are resistant to erythromycin are also resistant to clindamycin which is known as constitutive resistance (Drinkovic et al., 2001).

Although some *S. aureus* isolates are susceptible to clindamycin *in vitro*, they may not be effective *in vivo* particularly when the strain is resistant to erythromycin. This may be due to presence of inducible macrolide-lincosamide-streptogramin B resistance (iMLSB). The presence of inducible clindamycin resistance (iMLSB) can be detected in erythromycin resistant strains by the double disk diffusion assay (D test) (Somily and Babay,

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2008). The aim of this study was to determine the prevalence of inducible clindamycin resistance among clinical isolates of *S. aureus* by the phenotypic method in the tertiary care centre of north eastern India.

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology, Era's Lucknow Medical College and Hospital, Lucknow, India during a period of one year, from December 2008 to November 2009, in accordance with the ethical rules of Era's Lucknow Medical College and Hospital, Lucknow, India. It was a prospective cross sectional study.

Patient selection

A total of 260 isolates of *S. aureus* were isolated from various clinical samples, e.g. pus, blood, urine, body fluids, high vaginal swab, sputum throat swab, swab from surgical and non surgical wound tissue, and referred for bacteriological cultures from patients of all age groups and both sexes who were admitted in various inpatient departments of Era's Lucknow Medical College and Hospital. Isolates were presumptively identified on the basis of colony characteristics, Gram staining, catalase test, slide coagulase test and confirmation was done by tube coagulase test, modified Hugh and Leifson oxidation-fermentation (O/F) test, growth on mannitol salt agar and a DNase test (Baird, 1996).

In vitro antibiotic susceptibility and resistance pattern of *S. aureus* were carried out by the disc diffusion method of Kirby Bauer on Mueller Hinton agar using various drugs, including erythromycin (15 µg) and were screened for MRSA with 30 µg cofoxitin disc as per Clinical and Laboratory Standards Institute (CLSI) guidelines, 2012: The plates should be incubated at 33 to 35°C for 16 to 18 h; strains showing a zone diameter of less than or equal to 21 mm should be considered as having mec-A mediated oxacillin resistance (CLSI, 2012).

Double disc diffusion test with erythromycin and clindamycin

All strains that were erythromycin resistant were tested for the presence of iMLSB resistance by double disc diffusion assay (D test) according to CLSI guidelines. The test was done on Mueller Hinton agar with clindamycin (2 µg) and erythromycin (15 µg) placed 15 to 20 mm apart (edge to edge) on the same plate. Blunting of the circular zone of inhibition around the clindamycin disc on the side facing the erythromycin disc indicated the presence of iMLSB resistance. We interpreted the results according to three phenotypes:

- 1) MS Phenotype - Staphylococcal isolates exhibiting resistance to erythromycin (zone size ≤ 13 mm) while susceptible to clindamycin (zone size ≥ 21 mm) and giving a circular zone of inhibition around clindamycin were labelled as having this phenotype
- 2) iMLSB Phenotype - Staphylococcal isolates showing resistance to erythromycin (zone size ≤ 13 mm) while being susceptible to clindamycin (zone size ≥ 21 mm) and giving a D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were labelled as having this phenotype.
- 3) Constitutive MLSB (cMLSB) Phenotype - this phenotype was labelled for those staphylococcal isolates which showed resistance to both erythromycin (zone size ≤ 13 mm) and clindamycin (zone size ≤ 14 mm) with a circular shape of zone of inhibition if any around clindamycin.

RESULTS

In this study, among the 260 isolates, 105 were MRSA, while the rest 155 were MSSA (Table 1). Of all the 260 isolates, 39 were erythromycin resistant and clindamycin true sensitive and 60 isolates exhibited iMLSB phenotype (D test positive). In MRSA isolates, 47 exhibited iMLSB and 14 were true clindamycin sensitive. In MSSA isolates, only 13 exhibited iMLSB and 25 were true clindamycin sensitive. Constitutive resistance was 8.6% in MRSA and 4.5% in MSSA isolates.

DISCUSSION

cMLSB strains are easily recognized as resistant to both macrolides and clindamycin. The problem is that iMLSB resistance is not readily detected by standard *in vitro* susceptibility testing methods, unless they include measures that result in induction of clindamycin resistance. Such strains appear to be resistant to macrolides but susceptible to clindamycin under standard testing conditions. Various studies have reported prevalence of erythromycin induced clindamycin resistance in India (Table 2)

In this study we found a high prevalence of 44.2% of erythromycin resistance among *S. aureus* isolates. Overall, 99 (23.2%) isolates were erythromycin resistance and clindamycin sensitive. And of these 99 isolates, 60(23%) were found to be inducible clindamycin resistant (D test positive); 45% were MRSA and 8.4% MSSA, while the rest were D test negative. Another study from India also showed a very high frequency of inducible resistance (63%) in erythromycin resistant clindamycin sensitive isolates 74% for MRSA and 45% for MSSA (Ajanta et al., 2008). These observations suggest that if the D test had not been performed, nearly two thirds of the erythromycin resistant isolates would have been misidentified as clindamycin sensitive, resulting in therapeutic failure. In this study, all MRSA isolates have lesser prevalence of constitutive resistance (8.6%) than other studies. This was in agreement with the finding of Deotale only (Deotale et al., 2010). In MSSA isolates, the prevalence of constitutive resistance was 4.5% and inducible resistance was 8.5% which are within the limits of other studies (Table 2). The true sensitivity to clindamycin can only be judged after performing the D test on the erythromycin resistant isolates. Use of D test in a routine laboratory will help in guiding the clinicians regarding the judicious use of clindamycin in skin and soft tissue infections; as clindamycin is not a suitable drug for D test positive isolates but can be a drug of choice in the case of D test negative isolates.

Conclusion

In India, most of the laboratories do not include the D test

Table 1. Distribution of isolates.

Susceptibility pattern	MRSA (n = 105)	MSSA (n = 155)	Total = 260
MS phenotype(E resistant and CL susceptible with D test negative)	14 (13.3%)	25 (16.1%)	39 (15%)
Inducible MLS _B phenotype((E resistant and CL susceptible with D test positive)	47 (44.8%)	13 (8.4%)	60 (23.2%)
Constitutive MLSB phenotype(E resistant and CL resistant)	9 (8.6%)	7 (4.5%)	16 (6.15%)
E sensitive and CL susceptible	35 (33.3%)	110 (71%)	145 (55.7%)

CLSI –Clinical and Laboratory Standards Institute.

Table 2. Various studies in India showing prevalence of inducible clindamycin resistance in *Staphylococcus aureus* isolates.

Author's name	MRSA			MSSA		
	Constitutive MLSB phenotype (%)	Inducible MLS _B phenotype (%)	MS phenotype (%)	Constitutive MLSB phenotype (%)	Inducible MLS _B phenotype (%)	MS phenotype (%)
Gadepali et al. (2006)	38	30	12	15	10	12
Angel et al. (2008)	0	64	12	0	5	25
Gupta et al. (2009)	46	20	16	10	17.3	37.3
Ciraj et al. (2009)	15.3	38	0	0	12.9	9.7
Vandana et al. (2009)	0.05	48.7	30.7	1.4	9.5	56.1
Shrestha et al. (2009)	44.4	39.7	11.1	2.7	0	13.7
Deotale et al. (2010)	7.3	27.6	24.3	0	1.6	4
Pal et al. (2010)	38.8	43.6	18.7	7.3	6.93	10.9
Prabhu et al. (2011)	16.7	20	13.3	6.2	6.2	6.2

in routine antibiotic susceptibility testing for *S. aureus*. We therefore recommend that every laboratory should consider the D test in routine antibiotic susceptibility testing for *S. aureus*.

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