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COMPARATIVE ANALYSIS OF INDUCIBLE CLINDAMYCIN RESISTANCE AMONG COMMUNITY AND HOSPITAL ASSOCIATED *Staphylococcus aureus* INFECTION IN TERTIARY CARE HOSPITAL MAHARASHTRA INDIA

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Abstract- *Introduction*: Increasing frequency of *Staphylococcus aureus* (*S. aureus)* infections and changing patterns in antimicrobial resistance have led to concern in the use of macrolide lincosamide streptogramin type B (MLS_B) antibiotics to treat such infections. However, their widespread use has led to an increase in the number of *S. aureus* strains resistant to MLS_B antibiotics. Clindamycin is an appealing option because of its proven efficacy, safety and convenience of parental and oral administration in patients. The possibility of inducible resistance to clindamycin is a concern and has left very few therapeutic options for clinicians. *Material and Methods*: A total of 100 *S. aureus* strains were isolated from various clinical samples and were tested for antimicrobial susceptibility testing by Kirby-Bauer disc diffusion method and detection of iducible MLS_B were detected by double disc diffusion D-zone test method. *Results*: Of the total 100 *S. aureus* strains all were sensitive to vancomycin and linezolid while 50% were resistant to Oxacillin. Of the total 100 S. aureus 68 % were resistant to erythromycin of that 30 were inducible clindamycin resistant showed D-zone test positive and 90% isolates were isolated from pyogenic infections. Of the total 30 % inducible clindamycin positive isolated, 10 (33.33%) were methicillin resistant *S. aureus* (MRSA) while 20 (66.66%) were mithicillin sensitive *S. aureus* (MSSA). Of the total inducible clindamycin positive isolates 7 (70%) isolates were HA-MRSA and 3(30%) were CA-MRSA. *Conclusion*: Double disc diffusion D- zone test should be used as a mandatory method in routine disc diffusion testing to detect inducible clindamycin resistance.

Keywords- inducible MLS B resistant, S. aureus, HA-MRSA, CA-MRSA

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Introduction

Staphylococcus aureus (*S. aureus*) is etiological agent of a divers number of diseases, including necrotizing pneumonia, septic arthritis and osteomyelitis. 90% of all infections are result of skin and soft tissue structure breaches [1]. Earlier *S. aureus* has been associated principally to hospital and health care associated infections. However over recent decades there has been an alarming increase in *S. aureus* infections associated with antibiotic resistance throughout the community. Methicillin resistance *S. aureus* (MRSA) strains were initially described in 1961 and emerged in the last decade as one of the most important nosocomial pathogens. Since then, the major attention was focused on the knowledge of the prevalence of MRSA and their antimicrobial profile to guide the treatment strategy for infections caused by these strain [2-4]. In India also, large number of studies were carried out on prevalence of MRSA and their susceptibility pattern to decide the antibiotic policy for appropriate empirical therapy for these infections. In recent times, infections due to MRSA have become increasingly common in community and previously in healthy people who lacked traditional risk factors for acquisition of MRSA [5].

Given that the majority of reported community associated MRSA (CA-MRSA) infections are skin and soft-tissue infections and clindamycin represents a superior option as clindamycin comes in both intravenous and oral formulations (with 90% oral bioavailability) and this drug distributes well into skin and skin structures. Clindamycin is also less costly than some of the newer agents that might be considered for these infections. Finally, clindamycin may be able to inhibit production of certain toxins and virulence factors in staphylococci [6-8].

One of the major concerns with regard to the use of clindamycin for CA-MRSA infection is the possible presence of inducible resistance to clindamycin [8]. Inducible clindamycin resistance is not detected by standard broth microdilution testing, automated susceptibility testing devices, the standard disk diffusion test, or E-test. Uncertainty about the reliability of susceptibility reports for clindamycin, as well as confusion over the clinical importance of this inducible resistance, has led some clinicians to avoid use of clindamycin for staphylococcal infections whenever erythromycin resistance is noted.

Erythromycin resistant staphylococci often have cross-resistant to macrolides (erythromycin, spiramycin, claritrhomycin, azithromycin) lincosamide (lincomycin, clindamycin) and sreptogramin type B

antibiotics designated MLS_B resistant. MRSA infections and its varying patterns in antimicrobial resistance have led to concern in the use of MLS_B antibiotics to treat such infections. However, their widespread use has led to an increase in the number of *S. aureus* strains resistant to MLS_B antibiotics. Data describing MLS_B - prevalence or clinical predictors of the presence of MLS_B (inducible MLS_B) among MRSA as well as MSSA isolates are quite limited in India [5-8]. Clinically, bacterial strains exhibiting MLS_B resistant have a high rate of spontaneous mutation to constitutive resistance and the use of non inducer antibiotics such as clindamycin can lead to the selection of constitutive mutants and may result in clindamycin treatment failure [6-9].

A therapeutic decision is not possible without the relevant clinical and microbiological data. The increasing frequency of MRSA as well as rising rate of inducible clindamycin resistance is a great concern of clindamycin treatment failures and this is where the D-zone test becomes significant. Hence knowledge of prevalence of MRSA and their antimicrobial profile with detection of inducible clindamycin resistant has become a paramount in the effective treatment of the life threatening nosocomial infections and community acquired infections caused by these pathogens.

Present study was designed to isolate and confirm *S. aureus* from various clinical samples by standard, conventional methods. To study the antibiotic susceptibility pattern of above isolates with special reference to phenotypic detection of MRSA and detection of MLS_B resistant by D-zone test method and to characterize MLS_B resistance in both hospital and community associated *S. aureus* isolates, including MRSA and MSSA.

Material and Methods

The present study was conducted in the Department Of Microbiology, Dr. D. Y. Patil Medical College, Hospital And Research Centre, Pimpri, Pune-41018, from July 2014-August 2014.

Ethical Statement

Study protocols were approved by the institutional ethics committee of Dr. D. Y. Patil Medical College, Pune Ethics DYPV/EC/205/14

Selection of Test Strains

Total of 100 *S. aureus* isolated from various clinical samples like blood, pus, wound swab, urine, CSF and body fluids were tasted.

Sample Processing

S. aureus isolates were identified by the standard conventional methods [10] and the antimicrobial susceptibility testing was performed by Kirby Bauers disc diffusion method for co-trimoxazole (25µg), Gentamycin (30µg), erythromycin (15µg), linezolid (30µg), tetracycline (30µg), and vancomycin (30µg) as per guidelines from Clinical and Laboratory Standards Institute (CLSI). Screening for Oxacillin resistance using Oxacillin (1µg) on (Muller-Hinton) M-H agar supplemented with 2% NaCl followed by overnight incubation at 35°C [5,10].

Phenotypic Detection of Inducible Resistance to Clindamycin by D-zone Test

The inducible Clindamycin resistance was performed by D- zone test using erythromycin (15 µg) and clindamycin (2 µg) discs as per CLSI (Clinical Laboratory Standard Institute) guidelines. To detect inducible Clindamycin resistant, organism to be tested were cultured on a M-H agar plate at a Mc.Farland concentration of 0.5 to eventually cover the agar surface. Clindamycin and erythromycin discs were placed in the centre of the plate separated by a distance of 15 cm between the edges. Plates were incubated at 37° C for 24 hr. [5,11]. Organism that shows a blunting or flattering of the clindamycin zone are considered D-zone test positive, those that show no flattering were D-zone test negative

Three different phenotypes will be interpreted as follows:

MS Phenotype

Isolates showing circular zone of inhibition around clindamycin (Zone size> 21mm) and resistance to erythromycin (Zone size <13 mm) was labelled as MS phenotype.

Inducible MLSB phenotype: Staphylococcal isolates showing resistance to Erythromycin (zone size <13 mm) and sensitive to Clindamycin (Zone size>21mm) giving D - shaped zone of inhibition around Clindamycin disc were labeled as Inducible MLSB phenotype.

Constitutive MLSB Phenotype

Staphylococcal isolates showed resistance to both Erythromycin (Zone size <13 mm) and Clindamycin (Zone size < 14mm) with circular shape of zone of inhibition if any around Clindamycin.

Quality Control

S. aureus ATCC 25923 were used as the quality control strain.

Medical records for the source patients were reviewed for the demographic information, history of prior hospitalisation, presence of major comorbid conditions (e.g. Diabetes mellitus, renal dysfunction, post-surgical status, malignancy, solid organ or stem cell transplantation, neutropenia, trauma or burn injury) and antibiotic exposure within the preceding year.

MRSA isolates were designated as HA - MRSA if the source patient had any of the following risk factors: a history of hospitalization, residence in a long term care facility (e.g. nursing home), dialysis, or surgery within one year to the date of specimen collection; growth of MRSA within 48 h or more after admission to a hospital, presence of permanent indwelling catheter or percutaneous device at the time of culture; or prior positive MRSA culture report. If none of the above risk factors were present, the isolates were considered CA – MRSA [1,2].

Observation and Results

A total of 100 *S. aureus* were collected prospectively. Among these, 50 (50%) were MRSA and 50 (50%) were MSSA. All strains of *S. aureus* were susceptible to vancomycin and linezolid while 90% strains were sensitive to tetracycline. Moderate susceptibility were recorded from cotrimaxazole and gentamycin *i.e.* 55% and 49% [Fig -1].

The presence of MLS_B was confirmed by using D test.

A blunt edge with otherwise clear zone of inhibition around clindamycin disc was observed in D-zone test positive strains [Fig-2]. The prevalence of MLS_B among all *S. aureus* isolates were 30% (30). Of the 30 MLS_B producers, 10 were from MRSA strains while20 were from MSSA strains.

Of the total 100 strains of *S. aureus* 68% strains were erythromycin resistant, of that 30% strains were inducible clindamycin positive. [Table-1]. Maximum inducible clindamycin producer strains were

from pus sample i.e. 27(90%) and only 3(10%) were from body fluids [Fig-3]. 14 *S. aureus* strains were isolated from blood samples, of that 10 samples were received from ICU patients and all strains were not inducible clindamycin producers.

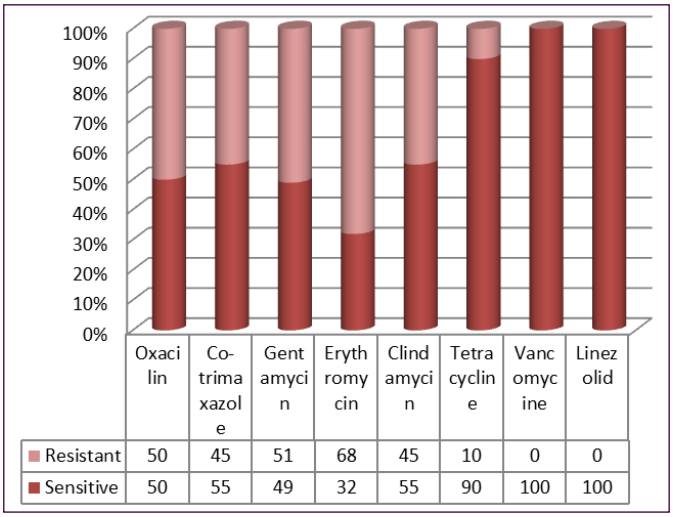

Fig. 1- Distribution of antimicrobial susceptibility of *S. aureus* (n=100)

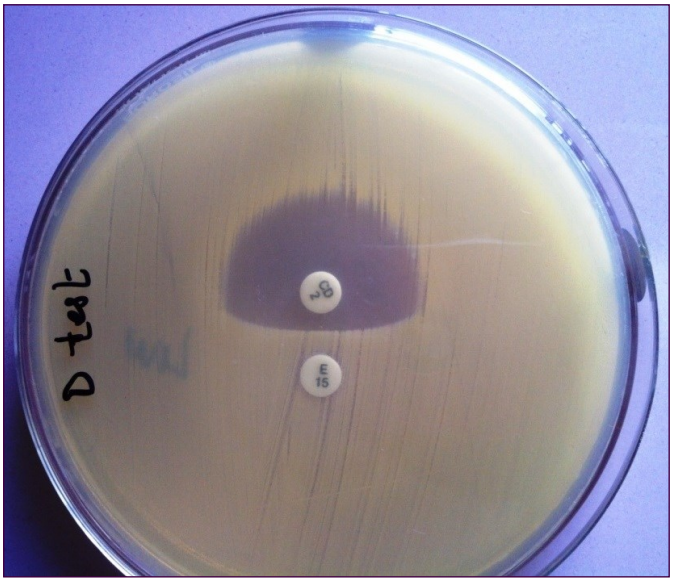

Fig. 2- Positive D-zone test

Table 1- Distribution of MRSA, MSSA and inducible clindamycin resistant (D Test positive) isolates from clinical samples

Specimen (no of samples)	MRSA isolates	MSSA isolates	D-test positive isolates
Pus (58)	28 (48.27%)	30 (51.7%)	27
Body fluids (28)	21 (75%)	07 (25%)	3
Blood (14)	01 (7%)	13 (92.8%)	0
Total (100)	50 (50%)	50 (50%)	30

Of the 30 MLS_B producers, 10 were from MRSA strains while20 were from MSSA strains [Fig-4]. Among 50 (MRSA) 20(40 %) were CA-MRSA and 30(60%) were HA MRSA. MRSA were predominantly isolated from adults in the age group of 18-59 years. The presence of MLS_B (total 30)was detected (07)70% in CA-MRSA and (03) 30% in HA -MRSA. The demographic and clinical characteristics of patients with *S. aureus* infections giving positive D- zone test results were obtained.

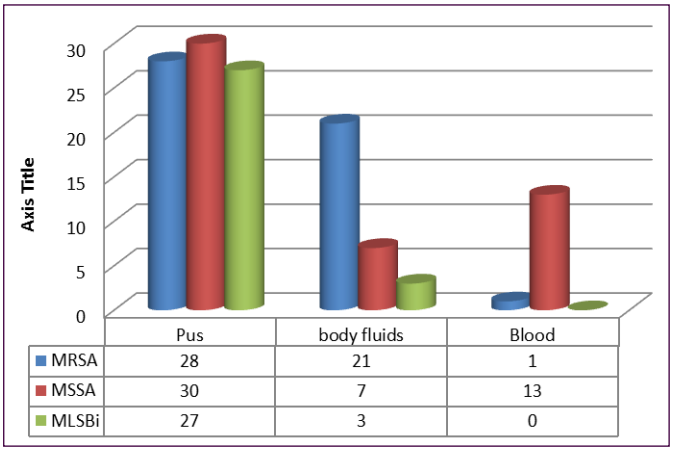

Fig. 3- Distribution of MLSBi from clinical samples

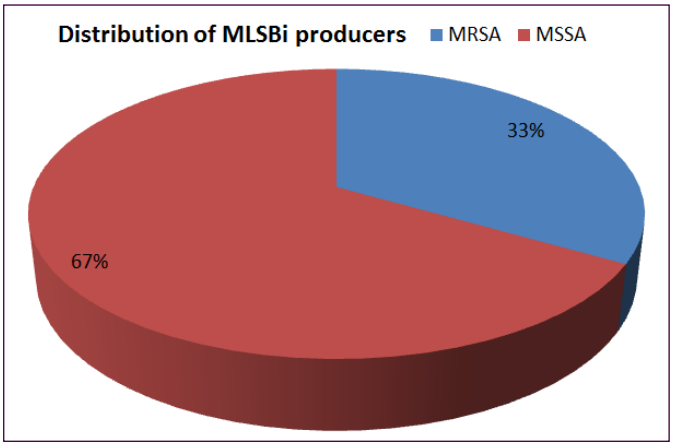

Fig. 4- Distribution of MLSBI in MRSA and MSSA strains

Discussion

Clinical data regarding the risk of emergence of inducible clindamycin resistance through selection of resistant mutants during therapy of MLS_B *S. aureus* infections are limited primarily to a few case reports. Much of the recent data have been derived from pediatric patients, because CA-MRSA was recognized early among some pediatric groups [13-14]. Although the data are not entirely conclusive, the trend has been that clindamycin treatment failures are more likely in MRSA infections due to MLS_B strains.

The present study was conducted to isolate and confirm *S. aureus* from various clinical samples by standard, conventional methods and also to characterize MLS_B resistance in both hospital and community associated *S. aureus* isolates, including MRSA and MSSA. 50 % *S. aureus* were oxacillin resistant while 68% strains were erythromycin resistant. Of the 68 erythromycin resistant strains, 30 (44.11%) strains were positive for D-zone test *i.e.* Inducible clindamycin resistant, while 17(25%) strain were MS- phenotype and 21(30.80%) were constitutive phenotype. In the present study, we have detected 20(66.66%) and 10(33.33%) inducible clindamycin resistant in MSSA and MRSA respectively. *S. aureus* remains the most prominent etiology of pyogenic infections. In the present study the majority of the isolates [58%] were from pus samples. Several reports also suggested that MRSA were isolated most commonly from pyogenic infections and ranged from 50% to 70% [8,15].

Studies on inducible clindamycin resistance from other parts of India ranged from 11% to 25% [16-18]. In the present study the overall inducible clindamycin resistance among *S. aureus* was demonstrated in 30 [30%] of isolates, In our study we observed that, almost all the CA-MRSA isolates were from pus samples and showed higher susceptibility to tetracycline, amikacin, clindamycin, linezolid in comparison to HA-MRSA. We also observed that, the multi-drug resistance and inducible clindamycin resistance was higher in HA-MRSA than CA-MRSA [19-21]. There have also been several studies reporting that clindamycin /lincomycin therapy failures in in sever staphylococcal infections dur to inducible clindamycin resistant strains [5-9] indicating that this has led to questioning the safety of clidamycin use against erythromycin resistant staphylococcal strains. However Lewis et al [21] and Levin [20] reported that clindamycin has been effective in some situations where inducible clindamycin resistance where demonstrated.

This may be useful in guiding the clinicians in treating CA-MRSA infections, as these are generally less resistant than HA-MRSA. However, further study needed for genetic analysis in detection of specific genes, other virulence markers and typing of SCC*mec* in MRSA. Since the main source of MRSA are the hospitals, the effective infection control measures and proper hand washing practices must be adopted by all the health-care providers to prevent spread of MRSA and MDR *S. aureus*. Screening of all the health- care providers for nasal carriage of MRSA may be done as a mandatory procedure in all the hospitals and treating carriers with topical mupirocin, may reduce MRSA and their spread in those setups. At the laboratory level, detection of MRSA by routine antibiotic susceptibility test must be done at the earliest for the better treatment outcome of patients, to control the infection and prevent their spread.

Conclusion

Due to of increasing prevalence in infections caused by MDR staphylococcal infections, it is decisive to do accurate drug susceptibility testing in addition to detect inducible MLS_B resistance by D- zone test. All clinical microbiological laboratories should implement of D-zone test if local prevalence is found to be considerable with isolates which exhibit MLS_B reported as being clindamycin resistant. Non MLS_B infections can be treated with clindamycin if appropriate.

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Conflicts of Interest: None declared.

References

[1] David M.Z., Cadilla A., Boyle-Vavra S. & Daum R.S. (2014). *PloS one*, 9(4), e92760.

[2] Naimi T.S., LeDell K.H., Boxrud D.J., Groom A.V., Steward C.D., Johnson S.K., Besser J.M., O'Boyle C., Danila R.N., Cheek J.E., Osterholm M.T., Moore K.A. & Smith K.E. (2001) *Clinical Infectious Diseases*, 33(7), 990-996.

[3] Duckworth G.J. (1993) *British Medical Journal*, 307(6911), 1049 -1052.

[4] Fluit A.C., Wielders C.L.C., Verhoef J. & Schmitz F.J. (2001) *Journal of Clinical Microbiology*, 39(10), 3727-3732.

[5] Fiebelkorn K.R., Crawford S.A., McElmeel M.L. & Jorgensen J.H. (2003) *Journal of Clinical Microbiology*, 41(10), 4740-4744.

[6] Rao G.G. (2000) *Journal of Antimicrobial Chemotherapy*, 45(5), 715-715.

[7] Yilmaz G., Aydin K., Iskender S., Caylan R. & Koksal I. (2007) *Journal of Medical Microbiology*, 56(3), 342-345.

[8] Steward C.D., Raney P.M., Morrell A.K., Williams P.P., McDougal L.K., Jevitt L., McGowan J.E.Jr. & Tenover F.C. (2005) *Journal of Clinical Microbiology*, 43(4), 1716-1721.

[9] Jadhav S., Gandham N., Sharma M., Kaur M., Misra R.N., Matnani G.B., Ujagare M.T., Saikat B. & Kumar A. (2011) *Biomedical Research*, 22(4), 465-469.

[10] Kloos W.E. & Banerman T.L. (1999) *Staphlococcus and Micrococcus*, *Manual of Clinical Microbiology*, 7th ed., ASM Press, Washington DC, 264-282.

[11] Clinical and Laboratory Standards Institute (2007) *Performance standards for antimicrobial susceptibility tests: Seventeenth informational supplement*, approved standard 8th ed., CLSI document M2-A9, 27(1).

[12] Clinical and Laboratory Standards Institute (2007) *Performance standards for antimicrobial susceptibility testing for bacteria that grow aerobically*, approved standard, 6th ed. CLSI document M7-A7, 27(1).

[13] Hussain F.M., Boyle-Vavra S., Bethel C.D. & Daum R.S. (2000) *The Pediatric Infectious Disease Journal*, 19(12), 1163-1166.

[14] Masano H., Fukuchi K., Wakuta R. & Tanaka Y. (1992) *Postgraduate Medical Journal*, 69, S122-S125.

[15] Patel M., Waites K.B., Moser S.A., Cloud G.A. & Hoesle C.J. (2006) *Journal of Clinical Microbiology*, 44(7), 2481-2484.

[16] Deotale V., Mendiratta D.K., Raut U. & Narang P. (2010) *Indian Journal of Medical Microbiology*, 28(2), 124-126.

[17] Siberry G.K., Tekle T., Carroll K. & Dick J. (2003) *Clinical Infectious Diseases*, 37(9), 1257-1260.

[18] Lall M. & Sahni A.K. (2014) *Medical Journal Armed Forces India*, 70(1), 43-47.

[19] Navaneeth B.V. (2006) *International Journal of Infectious Diseases*, 10(2), 184-185.

[20] Levin T.P., Suh B., Axelrod P., Truant A.L. & Fekete T. (2005) *Antimicrobial Agents and Chemotherapy*, 49(3), 1222-1224.

[21] Lewis J.S. & Jorgensen J.H. (2005) *Clinical Infectious Diseases*, 40(2), 280-285.

[22] Diekema D.J., Pfaller M.A., Schmitz F.J., Smayevsky J., Bell J., Jones R.N. & Beach M. (2001) *Clinical Infectious Diseases*, 32 (2), S114-S132.

[23] Anupurba S., Sen M.R., Nath G., Sharma B.M., Gulati A.K. & Mohapatra T.M. (2003) *Indian Journal of Medical Microbiology*, 21(1), 49-51.