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Research Article

STUDY OF COMMUNITY AND HOSPITAL ASSOCIATED METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* WITH SPECIAL REFERENCE TO INDUCIBLE CLINDAMYCIN RESISTANCE IN A TERTIARY CARE HOSPITAL

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ABSTRACT

MRSA has been considered a major nosocomial pathogen in healthcare facilities but recently it has been observed emerging in the community as well. Clindamycin is a preferred therapeutic option in the treatment of both methicillin susceptible and resistant staphylococcal infections. The present study was aimed to determine the incidence of constitutive and inducible clindamycin resistance among Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) and Hospital-associated methicillin-resistant *Staphylococcus aureus* (HA-MRSA) isolates. A 600 staphylococcal strains were isolated from various clinical specimens. Antibiotic susceptibility tests were performed using standard method. Methicillin resistance was detected by cefoxitin (30 ug) disc diffusion test using Mueller-Hinton Agar. D-test was performed on all erythromycin resistant and clindamycin sensitive isolates to detect inducible clindamycin resistance. MRSA was documented in 28 % amongst 600 isolates of *S. aureus*. Out of these 64.66 % and 35.33 % isolates of *S. aureus* were hospital associated and community associated respectively. Among these, 216 *S. aureus* were resistant to Erythromycin, 61 isolates were MRSA. Out of these 42 (68.85 %) were HA-MRSA and 19 (31.14) were CA-MRSA. We observed 3 (15.78 %), 16 (84.21 %), 0 % were iMLSB, MS phenotype and cMLSB in CA-MRSA respectively. 18 (42.85 %) iMLSB, 21 (50 %) MS phenotype and 3 (7.14 %) cMLSB observed in HA-MRSA. Our study suggested that MLSB resistance in *S. aureus* should be under constant surveillance in every country and region. The D- test for detection of iMLSB resistance should be carried out routinely in laboratories so as to prevent therapeutic failures.

Keywords: MRSA, CA-MRSA, HA-MRSA, Clindamycin, D-test, iMLSB.

INTRODUCTION

Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates are increasingly frequent causes of skin, soft-tissue and invasive infections in many communities. Usually, MRSA has been considered a major nosocomial pathogen in healthcare facilities only, but in the past decade in some areas, it has been observed emerging in the community as well[1]. Community-associated methicillin-resistant *Staphylococcus aureus* (CA-MRSA) is now an established pathogen in many communities in the United States as well as in the world[2,3]. The emergence of resistance to antimicrobial agents among *Staphylococci* is an increasing problem. Emergence of MRSA has resulted in therapeutic alternatives to treat staphylococcal infections. *S. aureus* is increasingly recognized as a cause of hospital associated (HA) and community associated (CA) infections. The Macrolides (Erythromycin and Clarithromycin) and lincosamides (Clindamycin and Lincomycin) serve as alternative. Clindamycin is commonly used for the treatment of serious staphylococcal infections due to its excellent pharmacokinetic properties but sometimes treatment failures were reported during therapy.[4,5] Widespread use of these macrolide lincosamide – streptogramin B (MLSB) antibiotics has led to an increase in resistance to these antibiotics especially clindamycin, amongst staphylococcal strains.[6-7] Changing patterns in antimicrobial resistance have led to renewed interest in the use of MLSB antibiotics to treat such staphylococcal infections. However, their widespread use has led to an increase resistant to MLSB antibiotics in the *Staphylococcus* strains[8]. The incidence of invasive infections has been rising with emergence of CA-MRSA and HA-MRSA[9,10]. Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory test, as they appear erythromycin resistant and Clindamycin sensitive *in vitro* when not placed adjacent to each other. In such cases, *in-vivo* clindamycin therapy may failure were reported by many studies.[4,5]

Data of the presence of iMLSB among CA - MRSA and HA-MRSA isolates is quite limited in India especially in Maharashtra, India. The present study was undertaken to find out the proportion of HA-MRSA and CA-MRSA and detect iMLSB resistance in both hospital and community associated MRSA in our institute.

MATERIAL AND METHODS

Study was approved by institutional ethical committee (BVDU/DCH/IEC/2010-11/06). The patients and their respective isolates were classified according to 2 categories: (a) Community-acquired isolates (b) Hospital-acquired isolates according CDC criteria[11].

Definitions of CA-MRSA

Organisms were considered to be community acquired if the isolates were recovered within 48 hours of hospitalization. The Community acquired MRSA occurs in individuals in the community who are generally healthy and who were not receiving healthcare in a hospital or on an ongoing outpatient basis.

Definitions of HA-MRSA

The HA-MRSA is considered only when any MRSA which was isolated from a patient after 48 hours of hospitalization or from a patient with a history of hospitalization for surgery or a residence in a long term care facility within 1 year of the MRSA culture date.

A total 600 isolates of *S. aureus* were isolated from various clinical specimens like pus, wound swabs, blood and various aspirations received in the department of Microbiology, B.V.D.U.M.C and H. Sangli, Maharashtra, India. All the *Staphylococcus* species were identified by conventional microbiological methods including colony morphology, gram stain, slide coagulation and tube coagulation test and mannitol fermentation test; then subjected to susceptibility testing by Kirby – Bauers disc diffusion method on Muller Hinton agar plate using routine antibiotic discs as per CLSI guidelines. Methicillin resistance was detected using Cephoxitin (30-ug) on Muller-Hington agar followed by incubation at 37^0C.[12]

Detection of inducible Clindamycin resistance

The isolates which were found to be Erythromycin resistant were examined for inducible Clindamycin resistance using double disc approximation test (D test) as per CLSI guidelines. Erythromycin (15 ug) disc was placed at a distance of 15 mm (edge to edge) from Clindamycin (2-ug) disc on Muller-Hington agar plate. After overnight incubation at 37°C, flattening of zone (D shaped) around Clindamycin in the area between the two discs indicated inducible Clindamycin resistance.[12,13] Three different phenotypes were used for interpretation like as following manner.

- **iMLSB**

  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.

- **cMLSB phenotype**

  Strains that were resistant to both erythromycin and clindamycin were defined as exhibiting constitutive MLSB resistance

- **MS phenotype**

  Isolates sensitive to Clindamycin and resistant to Erythromycin but circular zone of inhibition around clindamycin (D test negative) was labeled as MS phenotype[14,31].

RESULTS

Total 600 non-duplicate isolates of *Staphylococcus aureus* obtained from different clinical samples like, pus, wound swab, urine, blood, body fluids were included in this study. MRSA was documented in 168 (28 %) and MSSA in 432 (72 %). Among these 388 (64.66 %) and 212 (35.33 %) isolates of *S. aureus* were hospital associated and community associated respectively. Among these, 216 *S. aureus* were resistant to Erythromycin, 61 isolates were MRSA. Out of these, 42 (68.85 %) were HAMRSA and 19 (31.14) were CAMRSA. All 61 strains were subjected to D test to detect iMLSB. We observed 3 (15.78 %), 16 (84.21 %), 0 %, iMLSB, MS phenotype and cMLSB in CAMRSA respectively. 21 (50 %) MS phenotype, 3 (7.14 %) cMLSB and 18 (42.85 %) iMLSB observed in HA-MRSA. Figure 1-3 shows iMLSB, cMLSB and MS phenotype.

DISCUSSION

The increasing prevalence of MRSA, treatment options for staphylococcal infections have become more limited and changing pattern in antimicrobial resistance have led to recent interest in the use of clindamycin therapy to treat such infections. A therapeutic decision is not possible without the relevant clinical and microbiological data. The increasing rate of MRSA with *in vitro* inducible clindamycin resistance raises a concern of clindamycin treatment failures, hence the D test becomes significant.[15,10] Clindamycin has good oral, bone and tissue penetrative nature, potential antitoxin affects tolerability as well as cost it has been an attractive and alternative option for treatment against both MSSA and MRSA.[9,16] Erythromycin – clindamycin disc approximation test (D test) is simple, reliable method to detect inducible Clindamycin resistance in erythromycin resistant isolates of Staphylococci.[9,17] iMLSB may not be detected if erythromycin and clindamycin discs are placed in nonadjacent positions.[18] *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 iMLSB are available and some of these patients developed constitutive resistance during therapy.[7,5,19] Resistance to macrolide, lincosamides, streptogramin B (MLSB) antibiotics, most commonly results from acquisition of erythromycin resistant methylase genes (erm gene) which encode enzymes that methylate the 23 sr RNA. Resistance to MLSB can be inducible or constitutive. Accurate drug susceptibility data of the infecting microbe is an essential step in making appropriate therapeutic decisions. The initial step before starting the antimicrobial therapy of infected individuals is performing the antimicrobial susceptibility testing for clinical isolates to avoid indiscriminate usage of antibiotics on trial and error basis. Determination of resistance to MLSB antibiotics will be beneficial in selecting the appropriate treatment for Staphylococcal infections. Commonest antibiotic is being preferred while treatment of these staphylococcal infections in case of failure to β-lactam antibiotics is clindamycin. The clinical failure of Clindamycin therapy has been reported before[18]. Macrolide induced Clindamycin resistance was observed among the clinical isolates of *S. aureus* since 1968 which could not be detected by the routine disc diffusion method from such isolates constitutive resistant mutants are emerged and results in treatment failure with Clindamycin *in vivo*. Now D test is used to detect iMLSB and cMLSB (Cross ref of [15,16]) In our study, out of total 600 isolates of *S. aureus*, 212 (35.33 %) and 388 (64.66 %) strains were isolated from community acquired and hospital acquired respectively by clinical definition. 216 (36 %) strains were resistant to erythromycin. Among these 61 strains were MRSA. Out of these 61 MRSA, 42 and 19 strains were HA-MRSA and CA-MRSA respectively. 60.65 % isolates of MRSA were sensitive to Clindamycin, against which it would be safe and appropriate to use Clindamycin or other Macrolides. It correlates with previous studies who have reported 57 % of susceptibility towards Clindamycin among MRSA stains.[20] In the present study, the prevalence of HA-MRSA was 68.85 % and CA-MRSA was 31.14 %. This finding is in concordance with the studies of Tandra Chadha *et al*. who showed the prevalence of CA-MRSA to be 20.6 % and HA-MRSA to be 79.4 %. Several authors have reported the prevalence of CA-MRSA ranging from 1 to 36 %.[21-23] Changing pattern of resistance of *S. aureus* makes its periodic surveillance mandatory.[24] We observed 34.42 % iMLSB in MRSA, which is correlates with study by P. Sreenivasulu Reddy *et al* who observed it in 46.2 % iMLSB in MRSA[11] and 15.78 % in CA-MRSA concoredence with Tandra Chadha , but less than Patel M *et al* reported 33 % CA-MRSA. We recorded 42.85 % iMLSB in HA-MRSA it is less than study of Patel M *et al* reported 56 %.[25,26] Our study suggests that iMLSB is more common in health care-associated (42.85 %) as compared to community-associated (15.78 %) *S. aureus* isolates. Vasanthi *et al* and Lt Col Mahima Lall also found same observation. The presence of iMLSB was detected in 40.9 % HA-MRSA and 23.3 % CA-MRSA by Lt Col Mahima Lall *et al*.[27,28] Different patterns of resistance to clindamycin observed in various studies may be due to different geographical region for study, age group, methicillin susceptibility pattern and from hospital to hospital. 0 (0 %) and 5 (7.35 %) Constitutive resistance is seen in CA-MRSA and HA-MRSA respectively in our study. Constitutive resistance in our study is seen in 4.91 % of total MRSA isolates which is in accordance with V. Deotale-2010 study (7.3 %). This is contrary to the one Indian study by Angel *et al* which did not observe this in any of the isolates.[29,30]

Table 1: Phenotypic Pattern of Inducible Clindamycin Resistance among MRSA and MSSA

Organism	Phenotype		
	iMLSB	cMLSB	MS Phenotype
CA-MRSA	3 (15.78 %)	0 (0 %)	16 (84.21 %)
HA-MRSA	18 (42.85 %)	3 (7.14 %)	21 (51.54 %)
Total MRSA	21 (34.42 %)	3 (4.91 %)	37 (60.65 %)

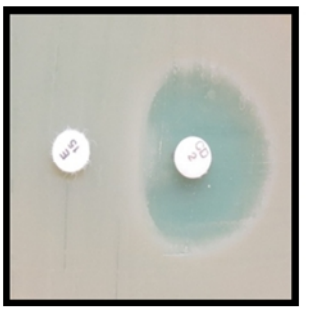

Figure 1: iMLSB

Figure 2: cMLSB

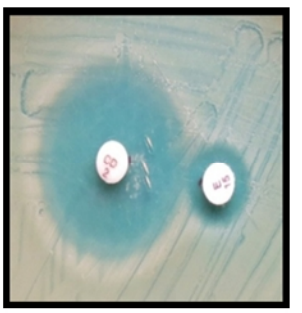

Figure 3: MS Phenotype

CONCLUSION

The reasons for the increasing incidence of MRSA in the hospitals and community could be multi factorial. Selection pressure due to overuse of antibiotics could have contributed to the emergence of these pathogens. The incidence of MLSB phenotypes and genotypes varies according to country, patterns of infections and drug use. It is suggested that MLSB resistance in *S. aureus* should be under constant surveillance in every country and region. The iMLSB resistance test should be carried out routinely in laboratories to prevent therapeutic failures. This study reflects the prevalence of iMLSB at our tertiary care center; however prevalence may differ from institute to institute.

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