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PREVALENCE OF INDUCIBLE CLINDAMYCIN RESISTANCE IN CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL IN BERHAMPORE , WEST BENGAL

Microbiology

Dr. Ranjan Basu M.B.B.S., MD (Microbiology), Assistant Professor, Dept. of Microbiology, Murshidabad Medical College, Berhampore, West Bengal, India

Dr. Mohua Bose* MBBS, MD (Microbiology), Associate Professor, Dept. of Microbiology, Murshidabad Medical College, Berhampore, West Bengal, India *Corresponding Author

Prof. (Dr.) Manash Sarkar MBBS, DTM&H, MD (Microbiology) Professor & Head, Dept. of Microbiology, Murshidabad Medical College, Berhampore, West Bengal, India

ABSTRACT

Introduction: Treatment failure of clindamycin therapy has been reported due to multiple mechanisms that confer resistance to macrolide, lincosamide and streptogramin antibiotics. . Moreover in vitro routine tests may fail to detect inducible clindamycin resistance due to *erm* genes. So detection of inducible clindamycin resistance among clinical isolates of staphylococcus aureus is of utmost clinical significance. **Materials and Methods:** The detection of Staph. aureus and MRSA was done using standard guidelines & inducible clindamycin resistance was performed by D-test using erythromycin and clindamycin discs as per CDC guidelines. .P value was calculated by SPSS v.18 software . **Results:** Among 184 Staph aureus, **54 (29.35%)** were MRSA and **130 (70.65 %)** were MSSA ; **76 (41.30 %)** were male and **108 (58.70 %)** were female ; **78 (42.40%)** were resistant to Erythromycin and **106 (57.60%)** were Sensitive to Erythromycin; Out of **106** Erythromycin sensitive Staph. aureus all were Clindamycin sensitive and **31 (16.84%)** were MRSA and **75 (40.76 %)** were MSSA. Among **78** Erythromycin resistant strain, **23 (12.5%)** were MRSA and **55 (29.89%)** were MSSA; MRSA showed **10 (5.43 %) $cMLS_B$, 1 (0.54 %) $iMLS_B$ & 12 (6.52 %) MS Phenotype** whereas MSSA showed **14 (7.61 %) $cMLS_B$, 16 (8.69 %) $iMLS_B$ & 25 (13.58 %) MS Phenotype. Conclusion:** Although several data available from developed countries, data from the developing countries are lacking. The study contributes to distinguish different resistance phenotypes in erythromycin-resistant *S. aureus* which helps to guide clinicians to formulate empiric therapy when culture reports are pending.

KEYWORDS

D test , MRSA , Inducible Clindamycin , Staphylococcus

Introduction :

Staphylococcus aureus is a notorious Gram positive organism responsible for various clinical infections and its emerging resistance to various drugs regularly throwing challenges to the clinicians .

Among different effective drugs , Macrolides, lincosamides and streptogramin (MLS) antibiotics are though structurally unrelated but still they are related microbiologically because of their similar mode of action. MLS antibiotics inhibit bacterial protein synthesis by binding to 23s rRNA, which is a part of the large ribosomal subunit. They have a spectrum of activity directed against Gram-positive cocci, Gram-negative cocci and intra-cellular bacteria such as chlamydiae and rickettsiae.[1]

Resistance to MLS antibiotics occur either through target site modification, efflux of antibiotics, or drug modification.[2] In target-site modification, methylation of the A2058 residue, located in the conserved domain V of 23s rRNA, takes place, which leads to cross resistance and results in the formation of the phenotype of resistance pattern known as MLS_B. This phenotype is encoded by erythromycin ribosome methylases(*erm*) genes that have been reported from a wide variety of microorganisms.[3]

The increasing prevalence of methicillin resistance among *Staphylococci* is also an increasing problem.[4] This has led to the renewed interest in the usage of acrolide–lincosamide–streptogramin B (MLS_B) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.[5] However, the wide spread use of MLS_B antibiotics has led to an increase in the number of *Staphylococcal* strains acquiring resistance to MLS_B antibiotics.[6]

The MLS family of antibiotics has three different mechanisms of resistance–target site modification, enzymatic antibiotic inactivation and macrolide efflux pumps. Clindamycin, a lincosamide antibiotic, is among the limited choice of antimicrobials effective against MRSA. There is concern about use of this antibiotic in the presence of Erythromycin resistance because of the possibility of induction of cross resistance among members of the macrolide, lincosamide, streptogramin B (MLS_B) group.[7] As MRSA infections have become increasingly common in the community setting ,the development of empirical antimicrobial therapeutic strategies for Staphylococcal infections has become more problematic .Clindamycin has long been an option for treating both MSSA and MRSA infections.

However, expression of inducible resistance to clindamycin could limit the effectiveness of this drug. Demonstration of inducible MLSB phenotype in isolates that are susceptible to Clindamycin and resistant to Erythromycin is possible by using Double Disk diffusion agar inhibitory assay or D – test. [8,9] Data describing MLS_B prevalence or clinical predictors of the presence of MLS_Bi among MRSA isolates are quite limited in India. In the present study, we aimed to characterize MLS_Bi resistance among Staphylococcus aureus including MRSA and MSSA isolates detected from patients' samples sent for culture and sensitivity from various OPDs & IPDs to our Microbiology department.

Materials and Methods :

This study was Conducted in the Department of Microbiology of Murshidabad Medical College & Hospital, Berhampore, Murshidabad , West Bengal for a period of May, 2016 to April 2018. A total of 184 Staphylococcus aureus isolates were recovered from the clinical samples received for culture and sensitivity in the microbiology Central laboratory of our hospital.

The isolates were identified by conventional methods using Gram stain , Catalase Test , Slide and Tube Coagulase tests . Detection of Methicillin Resistant Staph. aureus (MRSA) and Methicillin Sensitive Staph. aureus (MSSA) was done by using Cefoxitin disk as per CLSI guideline.[10,11]

D-Test

The Staphylococcus aureus isolates that were erythromycin resistant was tested for inducible resistance by the 'D test' as per CLSI guidelines. Erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from clindamycin (2 µg) on Mueller–Hinton agar plates previously inoculated with 0.5 McFarland bacterial suspensions. Plates were analyzed after 18 h of incubation at 37 °C. Interpretation of the inhibition zone diameters was as follows : If an isolate was erythromycin resistant and clindamycin susceptible, with a D-shaped inhibition zone around the clindamycin disc, it was considered to be positive for inducible resistance to Macrolide–Lincosamide–Streptogramin B (D test positive, $iMLS_B$ phenotype) [Figure–1]. If the isolate was erythromycin resistant and

clindamycin susceptible, with both zones of inhibition showing a circular shape, the isolate was considered to be negative for inducible resistance (D test negative, MS phenotype), but to have an active efflux pump. If the isolate was erythromycin resistant and clindamycin resistant, the isolate was considered to have the constitutive resistanceto Macrolide–Lincosamide–Streptogramin B ($cMLS_B$ phenotype). The quality control of the erythromycin and clindamycin disc was performed with *S. aureus* ATCC 25923.[12,13,14]

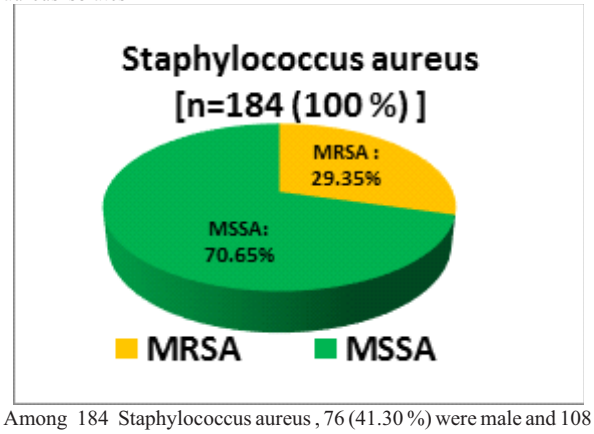
Statistical analysis

Demographic data were collected and SPSS version 18 was used for all statistical analysis. P value were calculated using Fisher's exact probability test using 2 X 2 / 2 X 3 contingency table as applicable and $P < 0.05$ was considered statistically significant.

Result :

Among various clinical samples received for culture and sensitivity in the Central laboratory Microbiology department of Murshidabad medical college , 184 Staphylococcus aureus isolates were detected. Out of 184 (n=184) Staphylococcus aureus, 54 (29.35%) were MRSA (Methicillin Resistant Staph. aureus) and 130 (70.65 %) were MSSA (Methicillin Sensitive Staph. Aureus) . (Chart -1)

Chart -1 : Distribution of MRSA & MSSA among Staylococcus aureus isolates



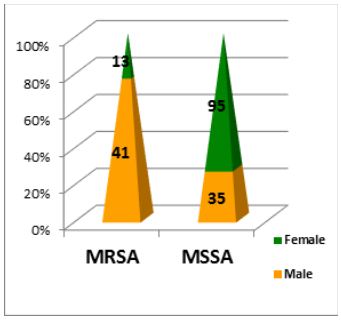
Among 184 Staphylococcus aureus , 76 (41.30 %) were male and 108 (58.70 %) were female ; out of 76 (41.30 %) male , 41 (22.28%) were MRSA and 35 (19.02 %) were MSSA whereas out of 108 (58.70 %)
female , 13 (7.07 %) were MRSA and 95 (51.63 %) were MSSA.

(Table-1, Chart - 2)

Table – 1 : Sex-wise distribution of MRSA & MSSA

Sex / Strains	Male	Female
MRSA 54(29.35 %)	41 (22.28 %)	13 (7.07 %)
MSSA 130(70.65 %)	35 (19.02 %)	95 (51.63 %)
Total	76 (41.30%)	108 (58.70 %)
P value < 0.0001 The outcome is statistically extremely significant		

Chart –2 : Sex-wise distribution of MRSA & MSSA



Among 184 Staphylococcus aureus, **78 (42.40%)** were resistant to Erythromycin and **106 (57.60%)** were Sensitive to Erythromycin. Out of 78 Erythromycin resistant Staphylococcus aureus, **23 (12.5%)** were MRSA and **55 (29.89%)** were MSSA. Among these 23 Erythromycin resistant MRSA strains **10 (5.43 %)** were pure Clindamycin resistant or Constitutive MLS_B phenotype **($cMLS_B$)** , **1 (0.54%)** was Clindamycin sensitive but D test positive or Inducible MLS_B phenotype **($iMLS_B$)** and **12 (6.52%)** were Clindamycin sensitive but D test negative **(MS Phenotype)** whereas among the **55 (29.89%)** Erythromycin resistant MSSA strains, **14 (7.61 %)** were pure Clindamycin resistant or Constitutive MLS_B phenotype **($cMLS_B$)**, **16 (8.69%)** were Clindamycin sensitive but D test positive or Inducible MLS_B phenotype **($iMLS_B$)** and **25 (13.58 %)** were Clindamycin sensitive but D test negative **(MS Phenotype) .** Out of **106** Erythromycin sensitive Staph. aureus all were Clindamycin sensitive and **31 (16.84%)** were MRSA and **75 (40.76 %)** were MSSA.**(Table-2)**

Table–2: Distribution of Clindamycin phenotypes among Erythromycin-resistant Staph. aureus

Phenotype	MRSA (%)	MSSA (%)	Total (%)
Erythromycin Sensitive & Clindamycin Sensitive	31 (16.84%)	75 (40.76 %)	106 (57.60%)
Erythromycin Resistant & Clindamycin Resistant(cMLSB)	10 (5.43 %)	14 (7.61 %)	24 (13.04%)
Erythromycin Resistant,Clindamycin Sensitive , D Test + (iMLSB)	1 (0.54 %)	16 (8.69 %)	17 (9.23%)
Erythromycin Resistant,Clindamycin Sensitive, D Test -(MS pheno)	12 (6.52 %)	25 (13.58 %)	37 (20.10%)
Total	54 (29.33%)	130 (70.67%)	184 (100%)
P = 0.0716			

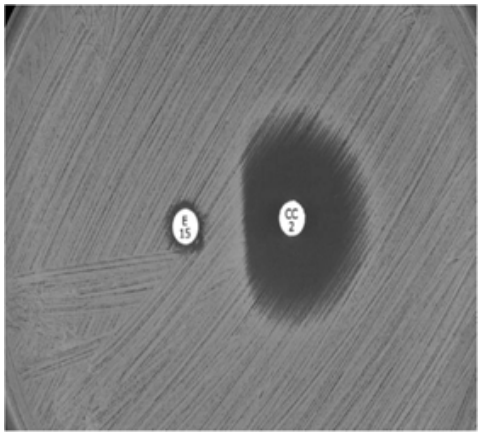


Figure-1 : Positive D Test

Discussion :

Clindamycin is an effective option to treat MRSA because of its proven efficacy, safety and convenience of parental and oral administration in patients. The possibility of inducible resistance is a great concern limiting treatment options for Staphylococcus aureus infection, both MRSA & MSSA. Clindamycin resistance can develop in Staphylococcal isolates with inducible phenotype and from such isolates, spontaneous constitutively resistant mutants have arisen both in vitro and in vivo testing during Clindamycin therapy.

Our study showed that out of 184 (n=184) Staphylococcus aureus, 54 (29.35%) were MRSA and 130 (70.65 %) were MSSA which closely corroborates with the study by Jadhav Savita Vivek et. al. which detected 32.51% MRSA and 67.48% MSSA[15] . Similar prevalence rate of MRSA was obtained from other studies in India namely 22.8 % by Pal and Ayyagari et.al.[16], 26.9 % by Shittu and Lin et. al.[17] and 26.6 % Mehta et al.[18] ; although lesser and higher percentage was obtained by other workers like 2.4 % by Pulimood et al.[19], 54.85% by Dar et al.[20]and 65 % by Borg et al.[21] The differences in the prevalence of MRSA among different countries and between different regions in a country could be due to difference in the study design, population, geographical distribution and the variation is probably due to differential clonal expansion and drug pressure in community. Further,

it emphasizes the importance of local surveillance in generating relevant local resistance data that can guide empiric therapy.[22]

Our study showed that MRSA are distributed more in males [41(22.28 %)] and less in females 13 (7.07%) whereas we got just reverse picture with MSSA showing more prevalence in females [95 (51.63 %)] and less in male [35 (19.02 %)] . The more male prevalence in MRSA may be due to more exposure to various antibiotics and geographical variation.

The incidence of MLS_B resistance varies significantly by geographical region. In our study, the prevalence of constitutive resistance, inducible resistance and MS phenotype was higher amongst MSSA revealing 7**.61%, 8.69%** and **13.58%** respectively when compared with MRSA which revealed **5.43%, 0.54%** and **6.52 %** respectively which closely corroborates with the finding by **B. Sasirekha et. al**. showing a prevalence of constitutive resistance, inducible resistance and MS phenotype among MSSA of **7.84%, 8.49%** and **13.07 %** respectively and among MRSA of **5.22%, 0.65%** and **5.88%** respectively.[23]

The outcome of our study was in concordance with a few other studies reported before. **Schreckenberger et al.**[24] and **Levin et al.**[25] showed higher percentage of inducible resistance in MSSA (19–20 %) as compared to MRSA (7–12 %). In our study, 8.69 % MSSA isolates were of the $iMLS_B$ phenotype, which is in concordance with the study by **Yilmaz et al**.[26] and **O'Sullivan et. al.**[27] who have found that 4–15 % of their MSSA isolates were of the $iMLS_B$phenotype.

As there is restricted range of antibiotics available for the treatment of methicillin-resistant *Staphylococcal* infections and the known limitations of vancomycin, clindamycin has the important role to treat such infections by Staphylococcus aureus. Further, using clindamycin, use of vancomycin can be avoided.[28] In addition, such studies can provide information about resistance to MLS_B phenotype group of antibiotics and can be useful for surveillance studies related to MLS_B resistance in *Staphylococci* and can contribute to predict empirical therapy to treat such infections.

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