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Identification of Macrolides Lincosamides and Type B Streptogramin (MLSB) Resistant Strains of *Staphylococcus aureus* in a Tertiary Care Centre at Thanjavur, India

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A B S T R A C T

Staphylococcus aureus is an important nosocomial pathogen responsible for mortality and morbidity in nearly most of the patients admitted in tertiary care centre because of its ability to induce methicillin resistance and its ability to show resistance to macrolides, lincosamides, type B streptogramin type and other groups of antibiotics resulting in treatment failure. MLSB strains whose expression can be constitutive (CMLSB) or inducible (iMLSB) is encoded by *ermA* and *ermC* genes. MLSB strains of *Staphylococcus* species were isolated in a tertiary care centre at Thanjavur from January 2018 to June 2018 by antimicrobial susceptibility testing (D test) and strains were confirmed by PCR. This study signifies the importance of early detection of resistant strains of *Staphylococcus* species at tertiary care centre to ensure appropriate management and strict antibiotic policies which reduces the patient mortality and morbidity due to *Staphylococcus* species infection.

Introduction

Staphylococcus genus consists of *Staphylococcus aureus* and coagulase negative staphylococci (CONS), *Staphylococcus aureus* is a gram-positive cocci arranged in clusters which is catalase and coagulase positive causes wide variety of diseases that includes diseases caused either due to direct infection and due to toxin secretion. Main pathogenicity is because of certain virulence factors that includes cell wall factors, surface proteins (protein A), enzymes and toxins (cytolytic toxins, panton valentine leucocidin, enterotoxin, TSST, epidermolytic toxin). The increasing prevalence of methicillin resistance in staphylococcal species is a growing problem. This has resulted in the use of macrolides, lincosamides and type B streptogramin antimicrobials for the treatment of staphylococcal infections. clindamycin, a lincosamide is used as treatment of choice for skin and soft tissue infections caused by staphylococcal species. clindamycin is also the antibiotic of choice in patients who shows intolerance to penicillin group of antibiotics.

Resistance mechanisms of *Staphylococcus* species apart from producing beta lactamases and is ability to alter penicillin binding protein by expressing *mecA* gene, also has resistant mechanisms which involves ribosomal target modification, affecting macrolides, lincosamides, and type B streptogramins characterizing the so called MLSB resistance. Its expression can be constitutive (CMLSB) or inducible (iMLSB) and is encoded by *ermA* (erythromycin ribosome methylase) and *ermC* genes, which are the main determinants for staphylococcal species resistance to macrolides, lincosamides and type B streptogramin antibiotics.

To detect inducible clindamycin resistance (iMLSB), tests recommended by CLSI (Clinical and Laboratory Standards Institute) is the double disc diffusion test (D test) & when there is such resistance CLSI recommends reporting them resistant to clindamycin.

In this prevalence study we identified *Staphylococcus aureus* species from various clinical isolates sent to department of microbiology during the period from January 2018 to June 2018 and samples were subjected to antimicrobial susceptibility testing using standard disc diffusion procedure and demonstrated various resistance patterns including MSSA, MRSA and MLSB.

Materials and Methods

Clinical Isolates

Clinical isolates received from microbiology laboratory in a tertiary care centre at Thanjavur from January 2018 to June 2018 was screened for resistance patterns of *Staphylococcus aureus*, we isolated 234 Staphylococcal species from various clinical isolates out of which 159 were methicillin resistant and 75 were methicillin susceptible, both of these strains were subjected to D test.

Mannitol salt agar (MSA) is used as a selective and differential medium for the isolation and identification of *Staphylococcus aureus*.

Deoxyribonuclease (DNase) test for the isolation and identification of *Staphylococcus aureus*.

Both mannitol salt agar (MSA) and DNase test improves the efficiency of tube coagulase test for identification of *Staphylococcus aureus*.

Antimicrobial susceptibility profile

The antibiogram was performed by disk diffusion technique in Mueller-Hinton agar, using antibiotic clindamycin 2µg, erythromycin 15µg, cefoxitin 30µg. the results were interpreted as standardized by CLSI.

Cefoxitin disk screen test

Cefoxitin is a second generation cephamycin antibiotic that induces the expression of *mecA* gene that codes for the altered penicillin binding protein (pbp2a). According to the standards determined by CLSI cefoxitin is used as a surrogate marker for *mecA* mediated oxacillin resistance. For the test, standard disk diffusion procedure, cefoxitin 30µg disk is placed in Mueller-Hinton agar plate incubated at 37°c for 16-18 hours and zone of inhibition less than 21 mm is considered as methicillin resistant.

D test

Staphylococcus aureus isolates with resistance to erythromycin and susceptibility or intermediate resistance to clindamycin in the antibiogram were selected. For the D test, a disk of 2µg of clindamycin was placed at a distance of 15-20mm from the edge of the disk of 15µg of erythromycin in Mueller-Hinton agar plate as shown in the picture.

After incubation at 35°c for 16-18 hours, isolates that shows no flattening of the inhibition are susceptible to clindamycin (negative D test) and isolates that shows flattening of the inhibition zone around the clindamycin disc indicates inducible clindamycin resistance (positive D test) as shown in the figure 4.

Molecular characterization

To confirm the presence of *mecA* gene and *ermA* gene, the isolated strains were subjected to molecular characterization by polymerase chain reaction (PCR).

After purification of bacterial DNA, the following steps were performed,
1) Initial denaturation: 95°c for 5 minutes.
2) Denaturation: 94°c for 30 seconds.
3) Annealing: 58°c for 30 seconds.
4) Extension: 72°c for 30 seconds.
5) Final extension: 72°c for 5 minutes.

Agarose gel electrophoresis was performed and it was viewed in UV transilluminator and the bands were observed.

Results and Discussion

Among 234 clinical isolates of *Staphylococcus aureus*, 159 (67.9%) strains were classified as MRSA (methicillin resistant *Staphylococcus aureus*) and 75 (32%) strains were classified as MSSA (methicillin sensitive *Staphylococcus aureus*) (Fig. 1 and 2; Table 1 and 2).

Table.1 Antimicrobial susceptibility profile of *Staphylococcus aureus*

PHENOTYPES	MRSAn (%)	MSSAn (%)
iMLSB(ERY-R, CD-S, positive D TEST)	34(21.3%)	13(17.3%)
cMLSB(ERY-R, CD-R, negative D TEST)	46(28.9%)	8(10.6%)
MRSA- methicillin resistant *Staphylococcus aureus*, MSSA – methicillin sensitive *Staphylococcus aureus*, iMLSB – inducible type, cMLSB– constitutive type, CD – clindamycin, ERY – erythromycin, R – resistant, S -susceptible		

Table.2 Various drug resistant strains of *Staphylococcus aureus*

RESISTANCE	DETECTION METHODS
MRSA – methicillin resistant *Staphylococcus aureus*	Cefoxitin disc diffusion method.
BORSA – borderline resistant s. *aureus*	Oxacillin screen agar
VRSA – vancomycin resistant s. *aureus*	Vancomycin MIC method or vancomycin screen agar or E TEST
VISA – Vancomycin intermediate s. *aureus*	MIC method or Vancomycin screen agar or E TEST
hVISA - heteroresistance VISA	E TEST
Inducible clindamycin resistance	D test

Fig.1 Yellow colonies of *Staphylococcus aureus* on mannitol salt agar (MSA)

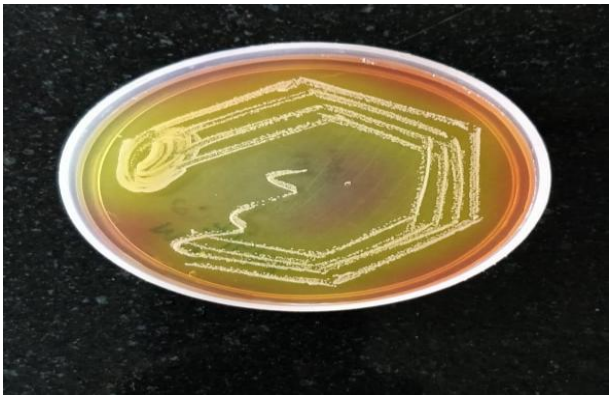


Fig.2 Zones around the bacterial colonies indicates positive DNase test

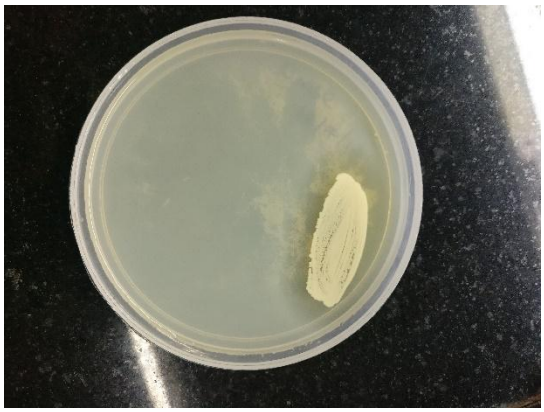


Fig.3 The small discs labeled E & C represent disks containing either 15 µg erythromycin (E) or 2 µg clindamycin (C) placed 15 to 20 mm apart

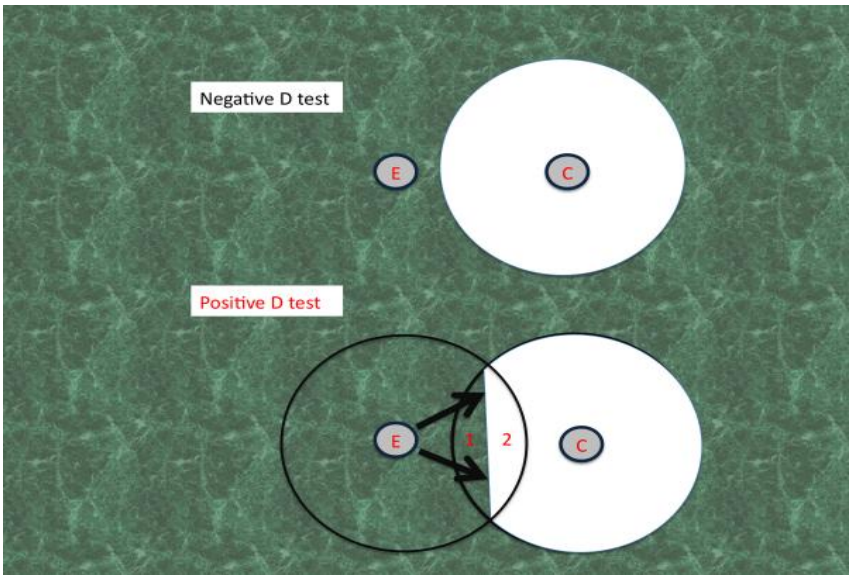


Fig.4 Positive D test, showing the flattening of the inhibition zone around the clindamycin disk adjacent to erythromycin disk (iMLSB phenotype)

Fig.5 Gel electrophoresis band pattern viewed in UV light following PCR for *mecA* gene confirming Methicillin resistant *Staphylococcus aureus*

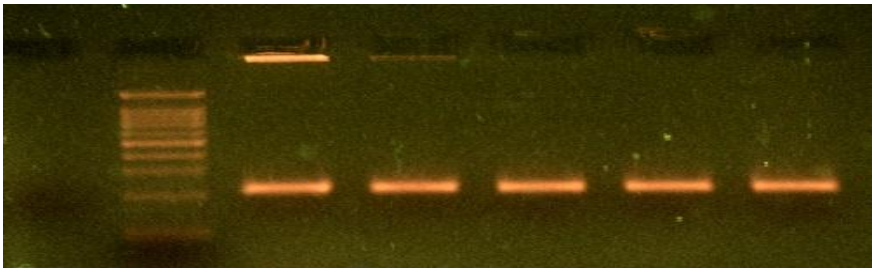
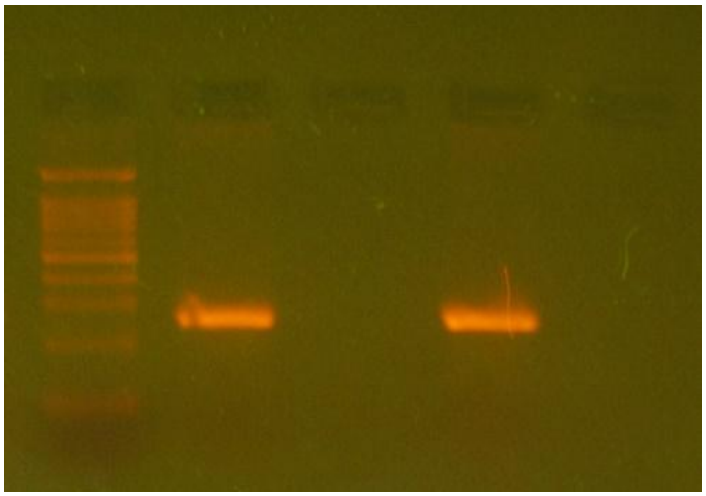


Fig.6 Gel electrophoresis band pattern viewed in UV light after PCR for *ermA* gene confirming inducible MLSB type of *Staphylococcus aureus*



Among which 34(21.3%) of MRSA strains and 13(17.3%) of MSSA strains were positive for D test which demonstrated iMLSB (ERY-R, CD-S, positive D TEST). 46(28.9%) MRSA strains and 8(10.6%) MSSA strains were exhibiting complete resistance to both erythromycin and clindamycin demonstrated cMLSB (ERY-R, CD-R, negative D TEST). In this prospective study constitutive type (cMLSB) type is more prevalent than inducible type (iMLSB) study also confirms that macrolides, lincosamides and streptogramin B (MLSB) antibiotic resistance is more prevalent in Methicillin resistant *Staphylococcus aureus* (50.2%) than methicillin sensitive *Staphylococcus aureus* (27.9%). This study was only able to demonstrate the presence of *ermA* gene among iMLSB type, *ermC* and *ermB* gene detection were not available during the course of the study.

Despite the lower frequency of iMLSB phenotype, it is necessary to perform routine D test in order to identify the resistance pattern and reduces the risk of antibiotic treatment failure in patients admitted in hospitals where there is higher prevalence of MRSA which results in higher case mortality and morbidity.

For adequate therapy it is important to identify the type of MLSB resistance since Staphylococcal species with constitutive resistance (CMLSB) shows *in vitro* resistance to all macrolides, lincosamides and type B streptogramins., whereas iMLSB type of Staphylococcal species can induce clindamycin resistance because it has an inducible methylase that methylates 23s component of the 50s ribosomal unit which is the binding site for macrolides, lincosamides and type B streptogramin (quinupristin), the methylase is encoded by a plasmid borne gene erm, this genotype has been associated with clindamycin treatment failure. In patients where resistance to macrolides, lincosamides and streptogramin B (MLSB) phenotype is detected, the choice of antibiotic will be vancomycin, daptomycin, teicoplanin and linezolid (Fig. 4 and 5).

In conclusion, *Staphylococcus aureus* being a super bug and its ability to show resistance to virtually all licensed antibiotics makes it very difficult to treat hospital acquired and community acquired staphylococcal infections, this study emphasize the resistance pattern of *Staphylococcus aureus* to macrolides, lincosamides and streptogramin B type (quinupristin) and its detection by a simple D test. This study also emphasize the fact that strict antibiotic policies and rapid identification methods is the need of the hour because of these super bugs developing resistance to various group of antibiotics thus making it mandatory in all hospital and laboratory practices. This can be helpful in the implementation of procedures that aims at controlling the spread of these kinds of antibiotic resistance patterns in hospitals.

References

CLSI, 2018, page 130 table 3E, page 136 table 3G

Jawetz, Melnick and Adelberg's medical microbiology 27th edition, page 203,210.

Koneman's color atlas and textbook of diagnostic microbiology, 7th edition, page 695, page 701.

MICRONS microbiology simplified Malathi Murugesan, page 62, table 3.

Prescott's microbiology ninth edition page 189.

The Brazilian journal of infectious diseases http://dx.doi.org/10.1016/j.bjid.2016.03.003

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