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MOLECULAR DETECTION AND SCCMEC ELEMENT MOLECULAR TYPING OF COMMUNITY-ACQUIRED METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS AT A TERTIARY CARE CENTRE AT NORTH INDIA.

Microbiology

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ABSTRACT

This prospective, cross-sectional study was conducted from October 2018 to April 2020, in the Department of Microbiology, at a tertiary care hospital. A total of 459 isolates were recovered from SSTIs, using strict aseptic precautions and processed as per standard microbiological techniques for the recovery of *S. aureus* based on staining characteristics, growth on Blood, MacConkey and Mannitol salt agar and in accordance with standard protocols. Disk diffusion method, Oxacillin screen Agar, HiCrome™ MRSA, MIC Determination by the E-test was carried out. DNA was extracted and PCR-based genotypic confirmation of *S. aureus* 16sRNA, mecA and PVL gene were carried out. The second multiplex PCR was done for sub typing the SCC mec elements. Out of 165 CA-MRSA isolates (n=157, 95%) had the presence of mecA gene and PVL gene was present in 68% (n=113). All the 165 CA-MRSA were typed by multiplex PCR out of which SCCmec type IVa was present in 26% (n=43), SCCmec type IVc was detected in 19% (n=32) and 38 (23%) isolates had the presence of SCCmec type V.

KEYWORDS

Staphylococcus aureus, CA-MRSA community-acquired MRSA, SSTI -skin and soft tissue infection, SCC- staphylococcal cassette chromosome, PVL- Panton-valentine leukocidin

INTRODUCTION

Staphylococcus aureus is a highly successful opportunistic pathogen. It is a ubiquitous colonizers of the skin and mucosa of humans and animals; *S. aureus* has an extraordinary capacity to adapt and survive in a great variety of environments. Staphylococci are nonmotile, non–spore forming, and usually catalase positive, and they are often unencapsulated. In humans, *S. aureus* has a niche preference for the anterior nares, especially in adults, and is shed onto healthy skin, including axilla and perineum. *S. aureus* can exist as a resident or a transient member of the normal flora. Nasal carrier rate varies from 10% to 40% in CA-MRSA and can produce a wide variety of diseases, the resistance gene sought is *mecA,* which encodes low-affinity penicillin-binding protein A (PBP2A).[1][2][3]

Panton-valentine leukocidin. PVL is a peculiar homologue of Hlg, which was originally reported in 1932 by Panton and Valentine encoded by two genes, *lukS* and *lukF,* which can assemble either between themselves or with the components of Hlg, thus producing chimera complexes. PVL is encoded by mobile phages, the prevalence rate of PVL is usually low (≤2%) in MSSA and is present in almost 100% of isolates of CA-MRSA associated with skin and soft tissue infection (SSTI) and severe hemorrhagic pneumonia in children and young adults.[1][2][3]

Resistance Island Staphylococcal Cassette Chromosome *mec*

MRSA contains one resistance island called *staphylococcal cassette chromosome* (SCC) *mec,* wherein *mec* is the genetic element that confers resistance to methicillin. SCC*mec* is an exogenous piece of DNA that may vary between 15 and 60 kb. The SCC*mec* critical genes are the recombinases *ccrA*/*ccrB* and *ccrC,* which can mediate mobilization of the whole element, and the *mecA* gene, which mediate and confers intrinsic resistance of MRSA to almost all β-lactams. Several types of SCC*mec* have evolved from more generalist SCC cassettes, which are structures able to integrate a variety of genes and are functional equivalents to integrons in gram-negative bacteria.[1][2][3]

SCC*mec* types I, II, and III were belong to HCA-MRSA. They harbor multiple resistance determinants, they have relatively large sizes (35–60 kb), and they are therefore difficult to mobilize. Types IV, V, and VI were associated with CA-MRSA. They are much smaller (about 15 kb) than their hospital congeners and carry multiple antibiotic-resistance genes. However, associated with other elements like PVL and multiple SE genes. SCCmec typing is important to understand the epidemiology and clonal relatedness of MRSA strains. Keeping this background, the present study was conducted to detect and molecular subtypes SCCmec in CA-MRSA strains isolated from our institution.

MATERIAL AND METHODS

Study design and setup

This prospective, cross-sectional study was conducted from October 2018 to April 2020, in the Department of Microbiology, and affiliated tertiary care hospital. A total of 459 isolates were recovered from **SSTIs** from different outpatients (one isolate per patient). Samples were collected from patients, using strict aseptic precautions and in were processed as per standard microbiological techniques for the recovery of *S. aureus* based on staining characteristics, growth on Blood, MacConkey and Mannitol salt agar and spot tests like catalase, slide and tube coagulase, bacitracin resistance, Furazolidine sensitivity, Deoxyribonuclease (DNase) Test, Phosphatase Test, Mannitol Fermentation Test and the modified Hugh and Leifson's (OF) test in accordance with standard protocols [4] and immediately processed without any delay. Inclusion criterion for the study was followed as per definition of CA-MRSA by CDC-2000.

Antimicrobial susceptibility testing

The antimicrobial susceptibility test was performed by the Kirby Bauer's disc diffusion technique on Cation adjusted Mueller–Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines.[5,6] The antibiotics tested were as follows (potency in µg/disc):penicillin (10 units), ciprofloxacin (5 µg), clindamycin (2 µg), erythromycin (15µg), cotrimoxazole (1.25/23.75 µg), linezolid (30 µg) vancomycin (30 µg) and netilmicin (30 µg). Methicillin resistance was detected using cefoxitin (30 µg) discs (Table 1).. All the discs were procured from Hi-Media Mumbai. The susceptibility patterns were graded as sensitive, intermediate and resistant as per CLSI guidelines.[5,6]

MIC Determination

Minimum inhibitory concentrations (MIC) of antibiotics were determined by the E-test (bioMérieux, Marcy l'Etoile, France) However, vancomycin resistance was detected by Vancomycin Screen Agar and E Test both.

Detection of MRSA by phenotypic methods

Disk diffusion methods.

Disk diffusion method technique on Cation adjusted Mueller–Hinton agar, as per Clinical Laboratory Standard Institute (CLSI) guidelines were performed for all isolates using oxacillin and cefoxitin disks. The turbidity of suspension was adjusted as per McFarland standard turbidity and both oxacillin and Cefoxitin disk were placed aseptically on the Mueller–Hinton agar and incubated at 35°C for 18 h. The inhibition zone for each antibiotic disks were measured and by referring to CLSI guidelines reported as MRSA or methicillin susceptible *S. aureus*. [5,6] All of antibiotic disks were provided from (Hi-Media Mumbai)

Oxacillin screen Agar

Muller-Hinton agar plates containing 4% NaCl and 6µg/ml of oxacillin were prepared. To perform the oxacillin screen test, a swab dipped in 0.5 McFarland's suspension of the isolate was deposited as a spot on the agar surface and it was incubated at 35 °C for 24 h. Plates were observed carefully in transmitted light for any growth. Any growth after 24 h was considered oxacillin resistant.[5,6]

HiCrome™ MRSA

HiCrome™ Rapid detection media for detection of MRSA was procured from Hi-Media laboratory Mumbai. Peptone provide the

essential nutrients along with carbonaceous, nitrogenous and Vitamin B complex nutrients. The chromogenic mixture in the medium specifically cleaved by Staphylococcus aureus to give green coloured colonies. With the addition of both MeReSa Supplement (FD229) and Cefoxitin supplement (FD259) in medium it was made selective for MRSA.

Quality control

The quality control strains including methicillin resistant *S aureus* (MRSA ATCC 43300), methicillin sensitive *S. aureus* (MSSA ATCC 25293), ATCC MRSA 33592, BAA-41, 42, 44, 1683 and MRSA BAA-2094 were used as positive and negative controls. These strains were procured from Hi-Media laboratory Mumbai.

DNA extraction and Molecular detection

DNA was extracted from the Confirmed strains of MRSA using the spin column method (QIAGEN; GmbH, Hilden, Germany) as per manufacturer's instructions. PCR-based genotypic confirmation of *S. aureus* 16sRNA, mecA and PVL gene were carried out on the isolates by using Gene Amp 9700 PCR System (Applied Biosystems, Singapore) (Table 2). The second multiplex PCR was done for typing and sub typing the SCC mec elements of MRSA. Eight pairs of primers were used including the unique and specific primers for SCC mec types (subtypes) I, II, III, IVa, IVb, IVc, IVd and V (Table 3).

MRSA ATCC 43300, MSSA ATCC 25293, ATCC MRSA **33592 for SCCmec: Type III,** ATCC MRSA **BAA-41 for SCCmec: Type II,** ATCC MRSA **BAA-42 for SCCmec: Type VI,** ATCC MRSA **BAA-44 for SCCmec: Type I,** ATCC MRSA **BAA-**1683 **for SCCmec:** Type IV a and *pvl* positive**,** ATCC MRSA **BAA-**2094**for SCCmec: Type V and pvl negative, mecA positive** were used as positive and negative controls for **Quality control**. PCR products were run on 1.5% agarose gel, stained with ethidium bromide visualized under UV light and photographed. Reactions were incubated at 95°C for 10 minutes followed by 35 cycles of 95°C for 15 seconds, 55°C for 30 seconds, and 72°C for 30 seconds. The final extension was at 72°C for 10 minutes. The amplicons were purified using QIAquick PCR purification kit (QIAGEN; GmbH,Hilden, Germany). The PCR products were visualized on a 1.5% agarose gel with ethidium bromide dye under a UV transilluminator. Sequences were compared with known sequences using the BLAST facility (http://blast.ncbi.nlm.nih.gov).

Statistical analysis

Statistical analysis was done using SPSS 16 software. The prevalence of resistance to each antimicrobial agent were recorded as percentage.

RESULT AND DISCUSSION –

A total of 459 Staphylococcus aureus isolates were recovered from **SSTIs** from different outpatients. (one isolate per patient). Out of A total of 459 recovered from OPD samples (CA-MRSA) 35.9% (n=165) were found to be methicillin resistant. Out of 165 CA-MRSA isolates (n=157, 95%) had the presence of mecA gene and PVL gene was present in 68% (n=113). All the 165 CA-MRSA were typed by multiplex PCR out of which SCCmec type IVa was present in 26% (n=43), SCCmec type IVc was detected in 19% (n=32) and 38 (23%) isolates had the presence of SCCmec type V. None of the CA-MRSA isolates harboured SCCmec types I, II, and III. 100% of CA-MRSA was resistant against Penicillin, Methicillin and Oxacillin. All these isolates showed 35% (n=58) resistant to Gentamicin, 38% (n=63) resistant to clindamycin, 42% (n=70) resistant to ciprofloxacin, 48% (n=79) resistant to Cotrimoxazole and 64% (n=106) resistant to erythromycin (Table 1). However, there was no isolate which was resistant to linezolid, Daptomycin, Teicoplanin and vancomycin (Table 1). All these resistant CA-MRSA resistant isolates had shown MIC**≤0.5 against** vancomycin while MIC**>4 (R) against clindamycin, erythromycin and oxacillin.** This study highlights both phenotypic and genotypic methods for the identification of CA-MRSA. The novel features were reporting of multidrug resistant CA-MRSA in communities. This study for the first time reported SCCmec type V, SCCmec type IVc and SCCmecIVa the prevalent circulating strain among CA-MRSA. There is an ongoing change in prevalence, resistance profile and epidemiology of CA-MRSA strains.

CONCLUSION -

The highlights of this study included compiled, simple and easy format for the identification, susceptibility testing by using both phenotypic and genotypic methods for CA-MRSA. The novel features were reporting of multidrug resistant CA-MRSA in communities. For the first time from the local community, this study had reported the prevalent circulating strain of Novel SCCmec type V, SCCmec type IVc and SCCmecIVa subtype.

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Conflict of Interest: The author state None to declare.

Table 1: Showing Antibiogram and resistance percentage of CA-MRSA strains

Antibiotics	Number Resistance	Percentage
Penicillin(10units)	165	100%
Erythromycin(15µg)	106	64%
Azithromycin(15µg)	106	64%
Co-trimoxazole (1.25/23.75µg)	79	48%
Clindamycin(2µg)	63	38%
Ciprofloxacin (5µg)	70	42%
Gentamicin (10µg)	58	35%
Netilmicin(30µg)	59	35%
Linezolid (30µg)	0	0%
Teicoplanin (30µg)	0	0%
Vancomycin(30µg)	0	0%

Table 2: Primer sequences used for the confirmation of S. aureus and detection of mecA and PVL gene.

Primer name	Target gene	Sequence	Amplicon size
mecA (F)	mecA	AAAATCGATGGTAAAGGTTGGC	533 bp
mecA (R)		AGTTCTGCAGTACCGGATTTGC	
mecA F	mecA	TCCAGATTACAACTTCACCAGG	162 bp
mecA-R		CCACTTCATATCTTGTAACG	
Staph (F)	16s Rrna	AACTCTGTTATTAGGGAAGAAC	756 bp
Staph (R)		CCACCTTCCTCCGGTTTGTCACC	
Luk-PV-1	LukS/ F-PV	ATCATTAGGTAAAATGTCTGGA CATGATCCA	433 bp
Luk-PV-2		GCATCAAGTGTATTGGATAGCA AAAGC	

Table 3: Primer sequences used for typing and sub typing MRSA strains.

Primer name	Target gene	Primer sequence	Amplicon size
SCCmec I F	SCCmec I	GCTTTAAAGAGTGTCGT TACAGG	613bp
SCCmec I R		GTTCTCTCATAGTATGAC GTCC	
SCCmec II F	SCCmec II	CGTTGAAGATGATGAAG CG	398bp
SCCmec II R		CGAAATCAATGGTTAAT GGACC	
SCCmec III F	SCCmec III	CCATATTGTGTACGATGC G	280bp
SCCmec III R		CCTTAGTTGTCGTAACA GATCG	
SCCmecIVa F	SCCmec IVa	GCCTTATTCGAAGAAAC CG	776bp
SCCmecIVa R		CTACTCTTCTGAAAAGC GTCG	
SCCmecIVb F	SCCmec IVb	TCTGGAATTACTTCAGC TGC	493bp
SCCmecIVb R		AAACAATATTGCTCTCC CTC	
SCCmecIVc F	SCCmec IVc	ACAATATTTGTATTATCG GAGAGC	200bp
SCCmecIVc R		TTGGTATGAGGTATTGCT GG	
SCCmecIVd F	SCCmec IVd	CTCAAAATACGGACCCC AATACA	881bp
SCCmecIVd R		TGCTCCAGTAATTGCTA AAG	
SCCmec V F	SCCmec V	GAACATTGTTACTTAAA TGAGCG	325bp
SCCmec V R		TGAAAGTTGTACCCTTG ACACC	

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