EMERGENCE OF MRSA AND VRSA FROM VARIOUS CLINICAL SPECIMENS IN TERTIARY CARE HOSPITALS OF VIDARBHA REGION, MAHARASHTRA

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Abstract

Over past two decades the prevalence of antibiotic resistance is completely alarming towards increased rate on pandemic. The present study was conducted to determine the prevalence of MRSA and VRSA in Maharashtra,Vidarbha region.

A total 1425 clinical specimens (like urine, blood, pus) were microbiologically analyzed for the infection with Staphylococcus aureus. Throughout the all samples 765 numbers of Staphylococcus aureusisolates confirmed including coagulase positive (CoPS) strains. Out of these 281(36.73%) multi drug resistant to methicillin and 41 (14.59%) resistance to vacomycin confirmation was done by E-test method.

In present study the incidence rate of MRSA in Vidarbha region was including Chandrapur 38.69%, Nagpur 36.27%, Amravati 38.35% andGadchiroli 34.95% respectively.

Molecular characterization of isolated MRSA and VRSA have been done by 16s rRNA gene sequencing method. Amplification of 16s rRNA gene sequencing and gene targeting for detection of mecA and vanA gene has been used for phylogenetic study. Present study conclude that the average prevalence rate of MRSA 36.73% and 14.59% of VRSA among MRSA respectively. Vancomycin, Amikacin and Nitilin were found to be the most effective antibiotics against isolated Staphylococcus aureus.MRSA and VRSA strains were positive for mecAandvan A gene. The isolated strain ChP04 MRSA positive caring mecA and ChP12 VRSA positive strains caring vanA genes.

Keywords: MDR, MRSA, VRSA, Mec A, Van A, Staphylococcus aureus

INTRODUCTION

During twain decennary it's alarming expands the resistance of antibiotic with disparate serious infection**.** Accidently the bacterial infection had decreased with the discovery of penicillin in 1940 until *Staphylococcus aureus* began assemble to β-lactamase, which demolish the penicillin β-lactam core ring (2)**.**This increase in resistance to approach drove the development of methicillin drugs, which are effectively resistant against many genetic variations of the β-lactamase enzyme. *Staphylococcus aureus* has long been known as one of the most virulent microbes and its ability to establish itself in human hosts. All bacteria and viruses have the capability of causing harm and evading host defenses, but *Staphylococcus aureus* is especially well suited due to these attributes are well developed in the bacterium. Specifically, MRSA isolates have been associated with nosocomial infection and rapidly expand resistance to multiple drugs classes, latterly different strain with idiosyncratic phenotypes have emerged in the community and the reservoir of community associated MRSA is quickly expanding community related pathogen are likely to cause life-threatening systematic infection. The genetic mechanism of Methicillin-resistant *Staphylococcus aureus* was identified as a gene called *mecA*that codes for specific methicillin-resistant transpeptidase PBP2a (Penicillin Binding Protein 2a). The gene is inserted in a mobile genetic element known as the *Staphyloccal* chromosomal

cassette (SCC *mec*). Nosocomial isolates have larger SCC*mec*, owing to the accumulation over time of integrated plasmid or transposons that contribute to the multidrug resistance.Methicillin resistant *Staphylococcus aureus* (MRSA) is now endemic in India. The incidence of MRSA varies from 25% in western India to 50% in South-India (14).Community associated MRSA (CA-MRSA) has been reported increasingly in India(15). MRSA infections are onerous to treat because of their resistance to many of the frequently used antibiotics such as tetracycline, macrolides and aminoglycosides. Some of these MRSA strains are resistant to even the most strapping antibiotics, including vancomycin. Vancomycins are the drugs of choice for the treatment of serious MRSA infection. The first vancomycin resistant *Enterococcus faecalis* (VRE) strain was reported in France in 1988 and caused great concern about the transmission of mobile genetic elements containing the *vanA* gene to *Staphylococcus aureus*(8,13)**.**The first strain of vancomycin intermediate *Staphylococcus aureus* (VISA) was reported in Japan in 1997(4). The first vancomycin-resistant *Staphylococcus aureus* (VRSA) strain was reported (vancomycin MIC>256 µg/ml; teicoplanin MIC 128 µg/ml) due to acquisition of the *vanA* operon was detected in 2002 from Michigan(9) and in the same year, a second VRSA strain was found in Pennsylvania. Subsequently, several other cases of VRSA were reported in the United State (7).

The aim of the present study was to identify the emergence of vancomycin-resistant *Staphylococcus aureus* (VRSA) and methicillin-resistant *Staphyloccusaureus* (MRSA) among *Staphyloccusaureus* isolated from tertiary care hospital in Vidarbha region, Maharashtra, India and to determine the sensitivity of these isolates to different antimicrobial agents. Further search is also conducted for the *mecA*and*vanA*gene in MRSA and VRSA strains (16).

MATERIAL AND METHODS

Clinical isolates

A total of 765 numbers of coagulase positive *Staphylococcus aureus*were isolates randomly from clinical specimens (like blood, urine and burn wound swabs) of admitted patients in tertiary care hospitals, in Vidarbha region between February 2016 to Jun 2018. The specimens were collected under sterile aseptic condition and transported immediately to the microbiology laboratory for processing. The study was done at Centre for Higher Learning and Research in Microbiology, Sardar Patel Mahavidyalaya, Chandrapur (M.S.), India. The study was designed and permitted by the Institutional Ethics Committee and a written informed consent was obtained from every individual before the specimen collection.

Isolation of Staphylococcus aureus

The isolates were identified by characteristic of colony morphology of *Staphylococcus,* round, raised, opaque colonies and β-hemolysis were observed on blood agar and yellow colored colonies of *Staphylococcus aureus* were obtained on Mannitol salt agar. As an important diagnostic procedure, Gram staining was done from well-developed colony on Mannitol salt agar plate. The identification of *Staphylococcus aureus* was further confirmed by Coagulase and DNas test.

Antibiotic susceptibility testing

The antibiotic resistance profile was determined by the Disc Agar Diffusion (DAD) technique using different antimicrobial agents; Amikacin (30 µg), Ceprofaxacin (5µg), Chloramphenical (30 µg), Erythromycin (15 µg), Gentamicin (10 µg), Lincomycin (2µg), Methicillin (30 µg), Netillin (30 µg), Norfloxacin (10 µg), Oxacillin (1µg), Penicillin G (10 U), Trimethoprime (5µg) , Tetracycline (30 µg), Tobramicin (10 µg), Vancomycin (30 µg) (Hi-media, Mumbai India) according to the guidelines recommended by Clinical and Laboratory Standards Institute (CLSI) (10). The standard *Staphylococcus aureus*strains NCIM 5522 and NCIM 5521 were used as reference strains (Centre for Higher Learning and Research in microbiology, Sardar Patel Mahavidyalaya, Chandrapur) for MRSA and VRSA.

Determination of Minimal inhibitory concentration (MIC)

Minimal inhibitory concentration (MIC) of methicillin and vancomycin was determined by E-test of disc diffusion method using CLSI guidelines (Class II Special Control Guidance Document, 2009). Briefly, Plates of Hi-sensitivity agar (Hi-media) was prepared with forming lawn of inoculums prepared using 18-24 h old culture was spotted with placing gradient strip 0.5 mcg/ml to > 265 mcg / ml of oxacillin and vanomycin on respectively. Plates were incubated overnight at 35^{o}C for 24h before assessing the visible growth (CLSI Performance standards for antimicrobial susceptibility testing

M100-S (latest edition); CLSI Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically, Approved Standard M7-A; CLSI Methods for Dilution Antimicrobial Susceptibility Tests of Anaerobic Bacteria Approved Standard M11-A (Latest edition).

Molecular Analysis

A total of 765 number of *Staphylococcus aureus* isolates confIrmed including coagulase positive strains. The isolates showing multiple antibiotics resistant to determine by Disc Agar Diffusion (DAD) and confirmed by E-test were selected for analysis and molecular detection of *MecA* and *VanA* gene. Various isolate were submitted to the*SaiBiosystem Privet Limited* Nagpur, Maharashtra, for the 16S rRNA sequencing and gene targeting sequencing.

Bacterial Genomic DNA Isolation

Extraction of bacterial genomic DNA has generally involved two major steps: breaking of cell wall, and the extraction and purification of genomic DNA. The genomic DNA is usually extracted with CTAB (cetyltrimethyl ammonium bromide) extraction buffer (Doyle and Doyle 1987) and then purified through phenol/chloroform extraction (12).

PCR based 16s rRNA gene amplification and Sequencing

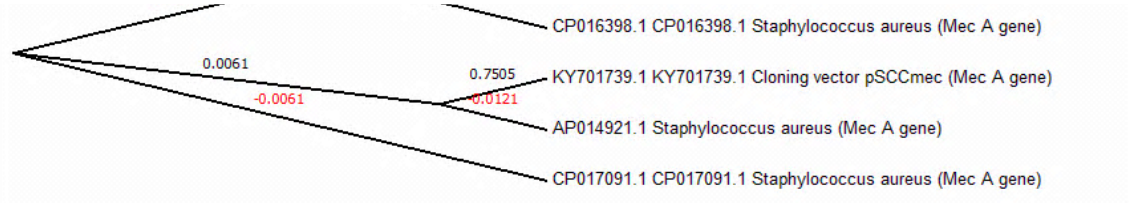
The PCR amplification of 16s rRNA gene of bacterial isolates were performed for the total of the 50µl reaction mixture. The amplification mixture comprised of 32 µl nuclease free water, 5.0 µl PCR buffer 10x, 2.0 µl dNTP (10 mM), 4.0 µl forward primer (10 µM), 4.0 µl reverse primer (10 µM), 1.0 µl Taq DNA polymerase enzyme (1U/ µl) and 200 ng DNA template. The temperature range for 50 µl each PCR reaction was programmed as: 58^0C, 57^0C, 56^0C, 54^0C, 52^0C, 50^0C, 48^0C. Scale up cycle sequencing was carried out at 54^0C using a thermal cycler (PTC 100, M J Research, Water Town, MA) at following condition: initial denaturation of 3 min. at 94^0C, denaturation of 1 min. at 94^0C, primer annealing for 1 min. at 54^0C, extension of 2 min, at 72^0C, final extension for 5 min, at 72^0C; total cycles and stored at 4^0C. The sequencing was carried out in ABI prim 3100 Genetic Analyzer (Applied Biosystems).

PCR amplification for mecA and vanA

The primer synthesis and PCR amplification of the*mecA* and *vanA* were done at SaiBiosystems Private Limited, Nagpur, using for DNA sequencing. PCR product was purified by Sanger chain method followed by cycle sequencing which was carried out Sanger's modified method of DNA sequencing and after post cycle sequencing purification, sequenced product were read by ABIprism 3100 genetic analyzer (Applied biosystems). The sequences were checked against microbial nucleotides database using BLAST search algorithm

Table No 1. Primers used to detect *MecA* and *VanA* gene and 16s rRNA gene sequencing

Primer name	Sequence details	Number of base	Ref.No
16s F	5' AGAGTTTGATCCTGGCTCAG 3'	20	16
16s R	5'AAGGAGGTGATCCAGCCGCA 3'	20	
***MecA* F**	5' GTAGAAATGACTGAACGTCCGCTAA 3'	25	11
***MecA* R**	5' CCAATTCCACATTGTTTCGGTCTAA 3'	24	
***VanA* F**	5' CATGAATAGAATAAAAGTTGCAATA 3'	25	
***VanA* R**	5' CCCCTTTAACGCTAATACGATCAA 3'	24	12



USA400-0051 (Accession number AP014921).

RESULTS AND DISCUSSION

Among 1425 clinical samples were collected and microbial analyzed for the infection with *Staphylococcus aureus.* Throughout the all samples 765 numbers of *Staphylococcus aureus*isolates confirmed including coagulase positive (CoPS) strains. Out of 765*Staphylococcus aureus* isolates were 281(36.73%) resistant to methicillin and 41 (14.59%) resistance to vacomycin subjected to antibiogram against 15 antibiotics by disk diffusion and resistance confirmed for all isolates with E-tests using strips of methicillin MIC of >265 mcg/ml and vancomycin MIC of >256 mcg/mlwas shown in [Figure **2]**. The number and percentage of isolation of MRSA and VRSA identified from different clinical specimen pus, urine and blood using conventional methodsgiven in**[Table 2].** *Staphylococcusaureus* isolated from both gender Male (57.29%) and Females (42.70%), respectively. The age wise incidence rate of MRSA include 0-10years (4.62%),11-20years (7.82%), 21-30years(11.74%), 31-40years (13.52%), 41-50years (27.75%), 51-60years (23.13%) and 61-70years (11.38%)**[Fugure].**

In current study particularly focus on Chandrapur, Nagpur, Amravati and Gadchiroli district area of vidarbha region. In these areas limited reports were available on development of methicillin and vancomycin resistant *S.aureus* from this part of India particularly Gadchiroli and Chandrapur district.In our study shown the incidence rate of MRSA in Vidarbha region was found to be Chandrapur 38.69%, Nagpur 36.27%, Amravati 38.35% and Gadchiroli 34.95% respectively. The average incident rate of MRSA infection in Vidarbha region was found to be 36.73%, most of the reports where it ranged between 20% to 50% in India.

In current reports which has given somewhat similar result, 38.56% MRSA where found in tertiary care hospital in New Delhi, India(18). The study of MRSA surveillance was conducted in early 1990s at three centres across India 31.8% MRSA prevalence was found in total 739 isolates of *S.aureus*(17).In current study, had shown the prevalence rate of VRSA in Vidarbha region including Chandrapur 11.88%, Nagpur 16.21%, Amravati 17.85% and 15.65% in Gadchiroli. The average prevalence rate of VRSA was found to be 14.59%.

All the selected isolated strain including ChP04, ChP15 and ChP12 are subjected to RT-PCT for *S.aureus* confirmation using universal primer. After 16s rRNA confirmed MRSA and VRSA isolates were further used for detection of *mecA* and *vanA* gene.

The phylogenetic study of ChP04 isolates sequence similarity 99% percentage with closely related with USA400-0051 strains which is prototype of USA400 strainsshown in [**figure1]**.

Both the selected MRSA and VRSA strains were found to be *mecA* and *vanA* gene. Various isolated MRSA and VRSA strains shows negative results may be due to mutation.

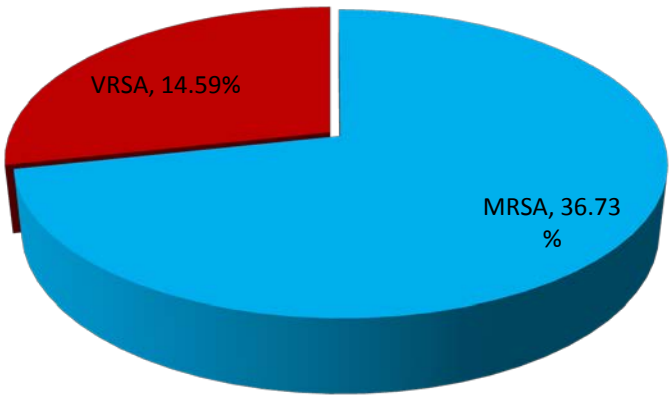


Figure: Overall distribution of MRSA and VRSA among MRSA strains.

Table No. 2: The Overall region wise distribution of Clinical sample (n=281)

Region	Three Different Kind of Sample			Total	
	Pus	Urine	Blood	No.	%
Chandrapur	62	25	12	99	35.23%
Gadchiroli	76	27	20	123	43.77%

Amravati	15	09	05	29	10.32%
Nagpur	21	05	04	30	10.67%
Total Sample	**174**(61.91%)	**66**(23.48%)	**41**(14.59%)	**281**	**100.0%**

Table No 3. : Distribution of VRSA strains among MRSA isolates in Vidarbha

Region	CoPSA	MRSA [n=281]		VRSA [n=41]	
		No.	%	No.	%
Chandrapur	261	101	38.69%	12	11.88%
Gadchiroli	329	115	34.95%	18	15.65%
Nagpur	102	37	36.27%	06	16.21%
Amravati	73	28	38.35%	05	17.85%
Total No.	**765**	**281**	**36.73%**	**41**	**14.59%**

Table No. 4: Age group distribution of patients having MRSA infection inVidarbha

Age Group	Male [n=161]		Female [n=120]		Total	
	%		%			%
00 – 10	10	6.21%	03	2.50%	13	4.62%
11 – 20	15	9.31%	07	5.83%	22	7.82%
21 – 30	12	7.45%	21	17.50%	33	11.74%
31 – 40	21	13.04%	17	14.16%	38	13.52%
41 – 50	46	28.57%	32	26.66%	78	27.75%
51 – 60	37	22.98%	28	23.33%	65	23.13%
61 – 70	20	12.42%	12	10.0%	32	11.38%
Total	**161**	**57.29%**	**120**	**42.70%**	**281**	**100.0%**

CONCLUSION

Staphylococcusaureus is a ubiquitous pathogenic microorganism, its ability to adapt to diverse environment forms and emerging as an important human pathogen can cause life-threatening infection globally.

In present study, concluded that isolated methicillin resistance *Staphylococcusaureus* shows prevalence rate in Vidarbha region include Chandrapur (38.69%), Nagpur (36.27%) Amravati (38.35%) and Gadchiroli (34.95%) and average prevalence rate of MRSA and VRSA was found in Vidarbha region36.73% and 14.59% respectively.

All the MRSA and VRSA isolates had shown multi drug resistance where MIC confirmed with E-test (CLSI, Approved Standard, M7-A, Latest edition). In these studies *S.aureus* isolated from both gender Male (57.29%) and Females (42.70%) respectively and incidence rate of was found to be 41-50 Yr (27.75%) adult age. PCR based amplification study shows the presence of *mecA and vanA*gene from MRSA isolates.

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