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PREVALENCE OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN WESTERN TAMILNADU

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ABSTRACT

Humans are natural reservoir for *Staphylococcus aureus*, and asymptomatic colonization is far more common than infection. Colonization by*Staph.aureus*may be persistent and can last for years. Recent reports of strains of Methicillin Resistant *Staphylococcus aureus* (MRSA) isolated from community have led speculation that the epidemiology of *S. aureus* is changing .Usually, MRSA infections havebeen a concern among hospitals for decades now and the reports revealthat the community acquired MRSA is increasing . The community acquired strains could possibly have arisen as a consequence of resistance gene transfer from a hospital acquired (nosocomial) donor into a susceptible recipient. With appropriate analysis of donor andrecipient chromosomes, it could be possible to determine whether these newly identified communityacquired strains are wild or self-supporting.The present study was conducted with a total sample of 1296 wound and other skin infection samples that were collected from different hospitals in western Tamilnadu. The specimens were inoculated in blood agar for isolation and identified as *S.aureus* by using standard method based on colony morphology, Gram's stain, catalase and coagulase test. A total 258 isolates were confirmed as S.aureus. These strains were processed by the following three techniques,(i) oxacillin, Methicillin and cefoxition disk diffusion method, (ii) cultured in MeRSA plate, (iii) Vitek 2 fully automated ID and AST system for detection mecA gene producer. 34 MRSA strains were identified out of 258 strains.

KEYWORDS: *Staphylococcus aureus, MRSA, mecA, Nosocomial, Antibiogram*

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INTRODUCTION

The genera *Staphylococcus* are normally present in normal skin or nasal colonizers of human beings[1]. Conventionally the *Staphylococcus* is divided into two groups on the basis of the coagulase reaction. The *coagulase-positive staphylococci* is the most commonly isolated pathogenic species *S aureus*[2]. The *coagulase-negative staphylococci* (CONS) are now known to comprise over 38 species. The CONS are common commensals of skin, although some species can cause opportunistic infections. Coagulase is a differential marker for *S aureus* but there is no direct indication as it is a virulence factor. Also, some natural isolates of *S aureus*are defective in coagulase. Variety of extracellular proteins and polysaccharides are secreted by *S. aureus*, few of which are virulent and result from the components expressed during infection. Usually antibodies will neutralize the toxins and enzymes secreted by staphylococcus. In many occasions both antibiotic therapy and surgical drainage are necessary to cure abscesses, large boils and wound infections. Staphylococci are common causes of infections associated with indwelling medical devices. These are difficult to treat with antibiotics alone and often require removal of the device. Some strains that infect hospitalized patients are resistant to most of the antibiotics used to treat infections. Glycopeptides are the only remaining antibiotics to treat the infections caused by Staphylococus aureus[1,3,4]. The antibiotic resistance is very common in *S aureus* and *few of CONS*. Methicillin resistance is indicative of multiple resistance. Methicillin-resistant *S aureus* (MRSA) causes outbreaks in hospitals among surgical patients and also now isolated from the community as well. Methicillin-resistant *Staphylococcus aureus* (MRSA) by definition harbors a gene, *mecA*, for methicillin resistance. The *mecA* gene codes for penicillin binding protein (PBP) 2a allows MRSA to continually synthesize its cell wall in the presence of β-lactam antibiotics[5]. The emergence of methicillin resistant *staphylococcus aureus (*MRSA) has posed a serious therapeutic challenge[6,7]. MRSA strains are difficult to eradicate as they are multi- drug resistant, leaving glycopeptides as the only drug of choice, resistance has been reported to those drugs also from various parts of the country[8, 9]. The knowledge of prevalence of MRSA and their antimicrobial susceptibility pattern is an absolute necessity for appropriate treatment and to avoid the cross infections. The present study was conducted to know the prevalence of MRSA in western Tamilnadu Hospitals. Laboratory diagnosis and susceptibility testing are crucial steps in treating and preventing MRSA infections. Therefore, methods used to detect MRSA in clinical samples should have high sensitivity and specificity and, most importantly, the result should be available within a short period of time. Various methods have evolved for rapid detection of methicillin-resistant staphylococci, but the optimal method for the detection remains controversial[10]. The most commonly used method in the laboratories is culture and antibiotic sensitivity test (AST) [Methicillin, oxacillin and Cefoxitin screen by disk diffusion (DD)[9-13]. Other methods available for diagnosing MRSA include mannitol salt agar (MSA) with oxacillin (agar screening method), minimum inhibitory concentration (MIC) tests by automated identification(ID) and AST system, agar dilution tests, commercially available MeRSA Agar etc[9-11,13]. All these tests are the conventional phenotypic methods of MRSA identification. Genotypic (molecular) method is the polymerase chain reaction (PCR) based method for detecting mecAgene, which remains the "gold standard" for diagnosing MRSA[9-13]. In this study three conventional phenotypic methods were used for Identifying MRSA strains;

- Disc Diffusion method (Methicillin, Oxacillin and Cefoxitin)
- Commercially available MRSA screening agar
- Automated ID and AST system (Vitek 2)

MATERIALS AND METHOD

A total of 1296 Pus & samples were collected from wound and other skin infection from different hospitals in western Tamilnadu, non-duplicated, for the period between January 2016 and October 2016.

Bacterial identification and antimicrobial susceptibility testing

The clinical specimens were inoculated on 5% sheep blood agar and MacConkey's agar (HiMedia), incubated at 37°C for 24 h, and examined for bacterial growth. S. aureus was identified using standard methods based on colony morphology, Gram's stain, catalase test, and coagulase test. A total of 258 isolates were confirmed as S. aureus. They were tested for methicillin resistance based on modified Kirby-Bauer disk diffusion method using oxacillin,

Methicillin and Cefoxitin disks on Mueller-Hinton agar[13] in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines using the criteria of standard zone sizes of inhibition to define sensitivity or resistance. The S. aureus strains were processed by following three techniques for diagnosing MRSA:

- Oxacillin, Methicillin and Cefoxitin Disc Diffusion method ,
- Culture in MeRSA medium containing oxacillin (at a concentration of 6 µg/ml of media) and 4% NaCl, and
- Vitek 2 Fully automated ID and AST system for the detection of possible mecA gene producer.

One MRSA ATCC (43300) and One MSSA ATCC (29213) were included in each batch of testing by all methods as a control.

STATISTICAL ANALYSIS

Statistical analysis was performed by GraphPad PRISM v5.0. All variables were recorded as number and percentage. Chi-Squre test was performed to find the specific association between groups

RESULTS

The Pus (Skin and Soft tissue infection) samples of 1296 collected from different hospitals in and around Coimbatore district, Tamilnadu, were processed in different bacterial culture media and it was found that 766/1296 (59.1%) samples showed positivity for different Gram Positive and Gram Negative Bacteria, of which 85/127 (66.9%) were from out patients and 681/1169 (58.3%) were from in patients (Table 1) (fig:4). Out of 766 positive cultures 258 Staphylococcus aureus strains were isolated and identified by using Catalase, Coagulase and Mannitol fermentation Biochemical tests. All the 258 strains were encompassed for MRSA study according to CLSI guidelines, using Oxacillin, Methicillin, and Cefoxitin screen (Figure 1 & 2). The result show that 34 (13.2%) MRSA strains were identified out of 258 S.aureus strains (Table 2) (Figure 3). All the strains had been confirmed for their Methicillin resistance by the expression of possible mec A gene by automated Vitek2 ID and AST system (Figure 4). District wise distribution of MRSA district showed that highest percentage of resistance was seen in Coimbatore (19%) compared to other areas (Table: 3, Figure 5).The antimicrobial activity was tested for selected regularly used anti staphylococcal antibiotics and Glycopeptides and Macrolides, the result showed 100% sensitivity (Table 4).

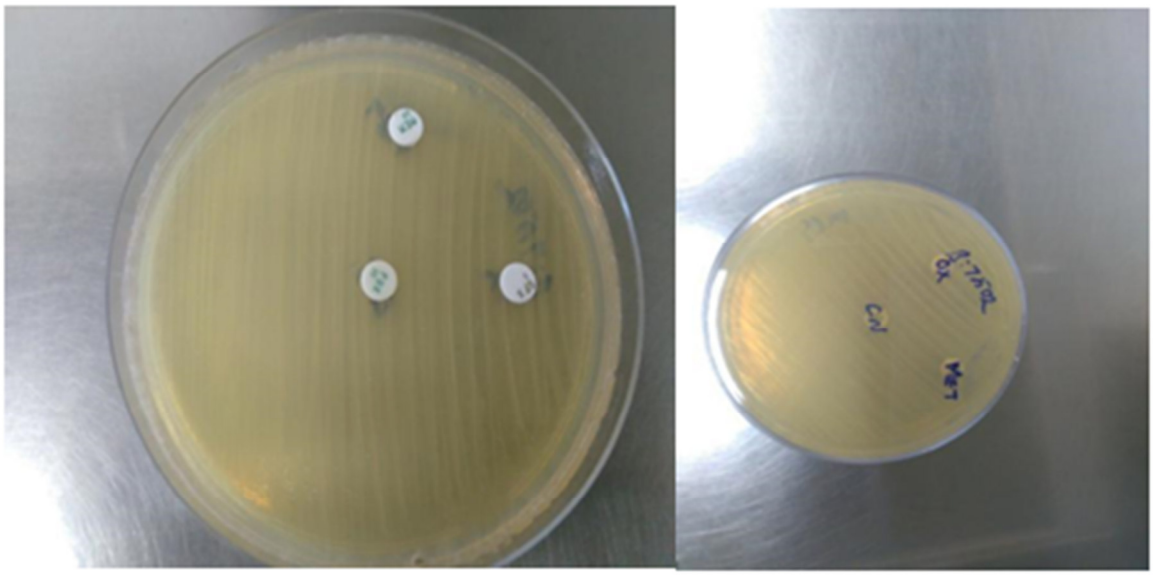


Figure 1
The resistance pattern of oxacillin (OX), cefoxitin (CN) and methicillin (M)

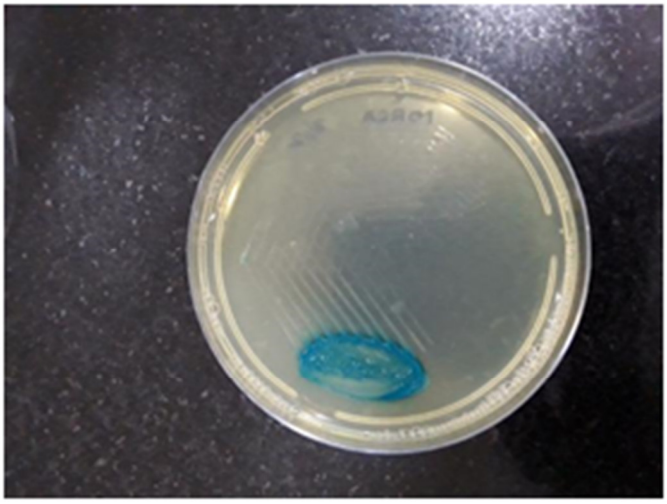


Figure 2
The appearance of greenish blue indicates the growth of MRSA in readily available MRSA plate.

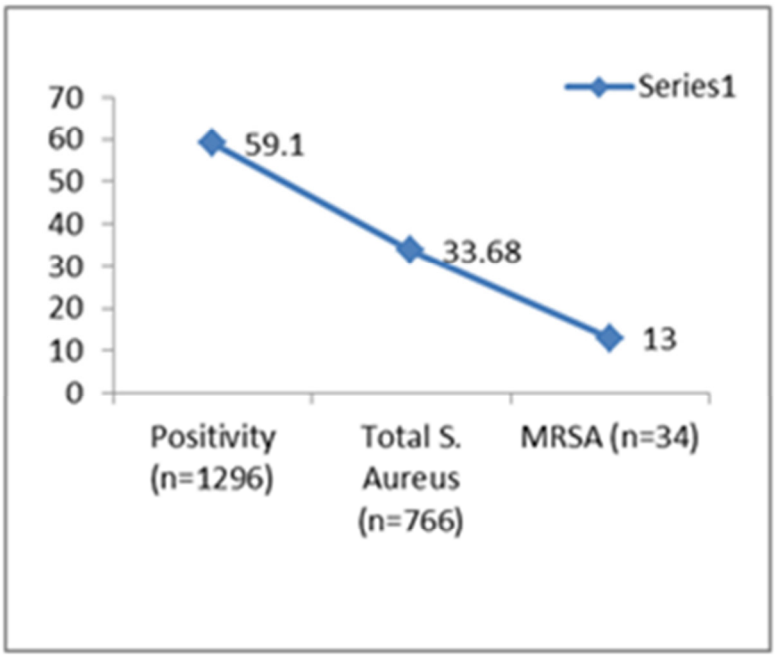
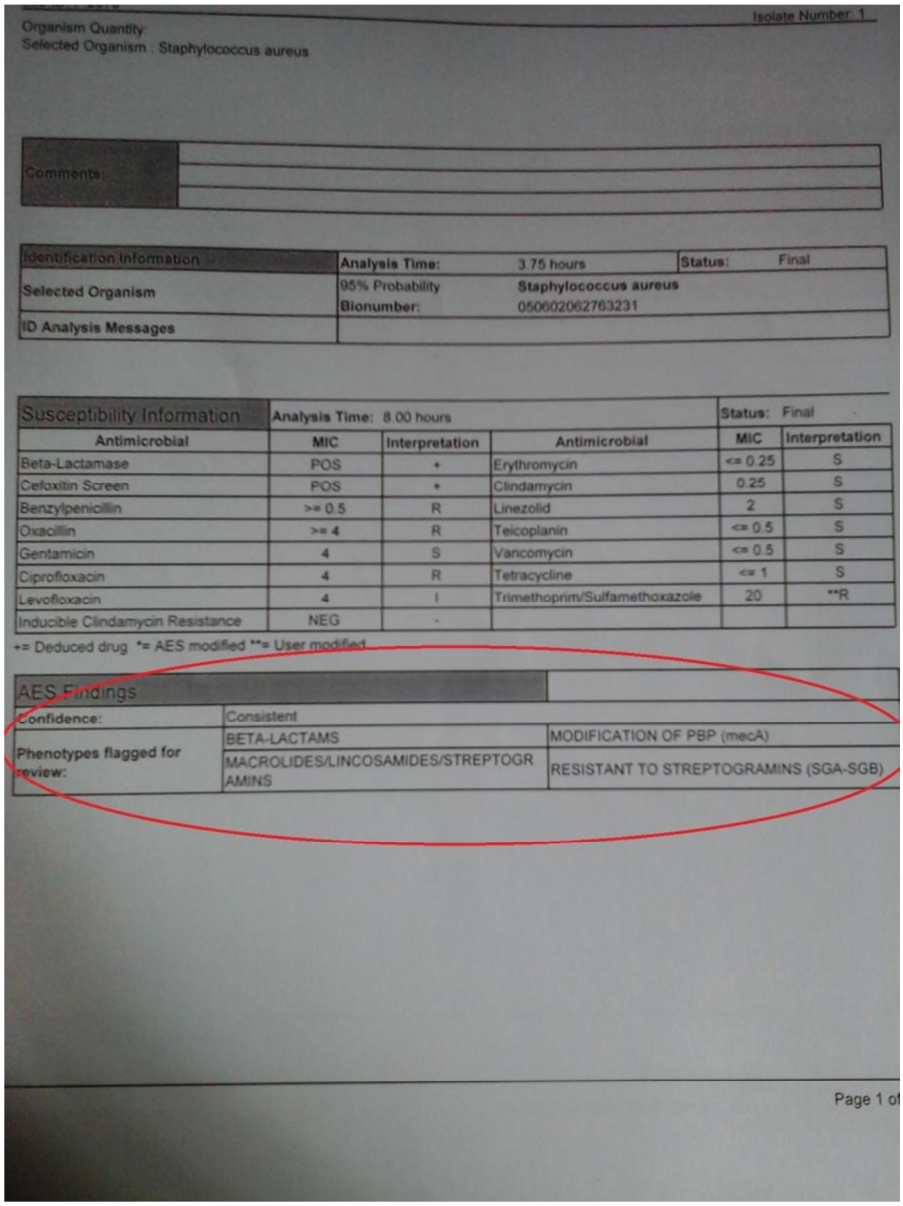


Figure 3
Percentage of culture positivity for S.aureus and MRSA



Isolate Number: 1

Organism Quantity:
Selected Organism : Staphylococcus aureus

Comments:

Identification Information	Analysis Time:	3.75 hours	Status:	Final
Selected Organism	95% Probability	Staphylococcus aureus		
	Bionumber:	050602062763231		
ID Analysis Messages				

Susceptibility Information	Analysis Time: 8.00 hours			Status: Final	
Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Beta-Lactamase	POS	+	Erythromycin	<= 0.25	S
Cefoxitin Screen	POS	+	Clindamycin	0.25	S
Benzylpenicillin	>= 0.5	R	Linezolid	2	S
Oxacillin	>= 4	R	Teicoplanin	<= 0.5	S
Gentamicin	4	S	Vancomycin	<= 0.5	S
Ciprofloxacin	4	R	Tetracycline	<= 1	S
Levofloxacin	4	I	Trimethoprim/Sulfamethoxazole	20	**R
Inducible Clindamycin Resistance	NEG	-			

+= Deduced drug *= AES modified **= User modified

AES Findings		
Confidence:	Consistent	
Phenotypes flagged for review:	BETA-LACTAMS	MODIFICATION OF PBP (mecA)
	MACROLIDES/LINCOSAMIDES/STREPTOGRAMINS	RESISTANT TO STREPTOGRAMINS (SGA-SGB)

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Figure 4
The VITEK 2 results indicates the detection of mecA

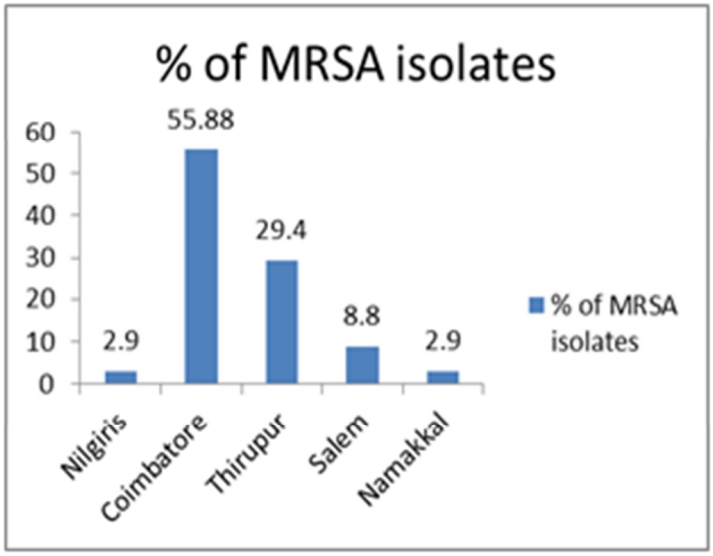

Figure 5
District wise distribution of MRSA in percentage

Table 1
Percentage of Positivity for S. aureus

S.NO	Samples from Out Patients	Samples from Admitted Patients	Positive Cultures - Out Patients	Positive Cultures - In Patients	Out Patient Positive%	In Patient Positive %
Total number	127	1169	85	681	66.9%	58.25%

Table 2
Percentage of Positivity for MRSA

sample type	Total sample	Positivity (n=1296)		Total *S.aureus* (n=766)		MRSA	
		no	%	no	%	no	%
PUS	1296	766	59.10	258	33.68	34	13

Table 3
District wise distribution of MRSA in percentage

S.NO	DISTRICTS	NO OF MRSA ISOLATES	
		No (n=34)	%
1	Nilgiris	1	2.90
2	Coimbatore	19	55.88
3	Thirupur	10	29.40
4	Salem	3	8.80
5	Namakkal	1	2.90

Table 4
Antibiogram of Methillin Resistant S.aureus (MRSA)

Name of Antibiotic	CA (n=18) (community acquired)	CA %	HCA (n=240) (Health care associated)	HCA %
Penicillin	11	61	213	89
Cefoxitin	11	61	213	89
Oxacillin	11	61	213	89
Methicillin	11	61	213	89
Gentamycin	18	100	240	100
Ciprofloxacin	17	94	232	97
Levofloxacin	17	94	232	97
Erythromycin	18	100	238	99
Clindamycin	18	100	238	99
Linezolid	18	100	240	100
Daptomycin	18	100	240	100
Teicoplanin	18	100	240	100
Vancomycin	18	100	240	100

CA- Community Acquired
HCA- Health Care Associated

DISCUSSION

Staphylococcus aureus has been known to cause complication both in community acquired and hospital acquired infection. S. aureus can lode in anybody site and can cause major complications. Longitudinal analysis shows, in industrialized countries, the incidence of S. aureus infection ranges from 10 to 30 per 100,000 person-years[14]. Between 1957 to 1990, the incidence of S. aureus infection has increased from 3 per 100,000 person-years in 1957 to 20 per 100,000 person-years in 1990[15]. In the setting we analyzed the prevalence of S. aureus was 19.9%. In Europe wide survey conducted to study the prevelence of S. aureus show a prevalence of 71%[16]. This stark difference could be due to the fact that, the region which we selected was not as industrialized as Europe and there are reports which state that, newly or emerging industrialized are has low S. aureus incidence[17]. The antibiotics that are developed against S. aureus targets, cell envelope, ribosome and nucleic acids. Methicillin belongs to the class of beta lactamase, which target cell envelope. The resistance to methicillin is developed by acquisition of genes which are not susceptible to drug action[18]. In this study, among 258 S. aureus positive patients, 34 (13.2%) are MRSA. This prevalence is very low compared to the study conducted in other settings in India such as Bangalore[19], Chennai[20]. The prevalence of MRSA in community acquired infections was 66.9% and hospital acquired was 58.3%. The prevalence of community acquired infection was quite high compared to hospital acquired infection. This is contrast to the reports from other settings, wherein the prevalence of community acquired infection was low compared to hospital acquired infection. However, the prevalence of hospital acquired MRSA is quite high compared to the data collected from all over India[21].

CONCLUSION

In this study, the MRSA prevalence of 13% may be considered to be high, the reason being that there has not been any previous epidemiological data on MRSA in that geographical zone. Although, MRSA prevalence is known to vary with geographical location, type of hospital, studied population and method of detection employed. However, on considering the clinical implication of MRSA infection and its rapid transmission capacity, periodic surveillance of MRSA strains needs to be done. Based on the periodic reports effective and efficient infection control and preventive measure has to be adopted.

CONFLICT OF INTEREST

Conflict of interest declared none.

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