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MRSA in Gymnasium Community

S. Pavithra[1], Prathiba Gnanasekaran[2], A. Subhashini[3]

[1,3]Ethiraj College for Women, Deprtment of Microbiology, 70, Ethiraj Salai, Egmore, Thousand Lights, Chennai, Tamil Nadu 600008

[2]Sathyabama Dental College and Hospitals, Sathyabama University, Rajiv Gandhi Salai, Jeppiaar Nagar, Chennai, Tamil Nadu 600119

Abstract: ***The antibiotic resistance of Staphylococcus aureus is increasing worldwide and remains as a potential medical threat. A total of 175 samples were collected randomly from 35 men and women who work out regularly in gymnasium. The samples were collected from sites such as palm, forearm, nose, shoe and towel (n = 175) from each individual. S. aureus were isolated and identified using cultural and biochemical methods and confirmed by coagulase test. Among the 175 samples, 9 isolates (25%) were positive for coagulase tube test. The Antibiotic sensitivity test (AST) for the isolated S. aureus strains were determined by Kirby Bauer method using appropriate antibiotics. The percentage of test cultures which showed resistance to methicillin (30µg), amoxycillin (30µg), penicillin (10 U), bacitracin (10 U), vancomycin (30µg), novobiocin (50 µg) and furazolidone (50 µg) were 86, 71, 71, 57, 57, 57, and 57% respectively. The percentage of methicillin resistant Staphylococcus aureus (MRSA) observed from various screening methods such as oxacillin agar screening test (6mg/l), cefoxitin disc screening test (30 µg), and minimum inhibitory concentration (MIC) - agar dilution method for the S. aureus isolates were 17% (n = 1). The MIC test using different concentration of oxacillin revealed one MRSA strain which had a MIC of ≥ 128 mg/l of oxacillin antibiotic and considered as highly resistant strain. A total of one MRSA strain has been isolated out of 175 samples, though it is not very high but shows the prevalence or contamination of CA-MRSA in gymnasium community. The study shows the importance of disinfection or handwashing before and after exposure to gymnasium since there is a risk factor for MRSA contamination. Further this study indicates Cefoxitin test (30µg) can be a used for detection of MRSA.***

Keywords: *S. aureus*, MRSA, gymnasium community, resistant strain

1. Introduction

Colonization of *S. aureus* plays a vital role in spreading of infection to the surface of human and it is the first step in disease establishment. The main site of *S. aureus* colonization includes skin and nose. *S. aureus* acquired from an external source could be the cause of an infection when inoculated into an open wound [19]. It also causes opportunistic infections in individuals with weak immune system; open wounds and children are at higher risk of infection and are vulnerable host to infection of *S. aureus.* The common infections caused by *S. aureus include* skin and soft tissue, musculoskeletal, respiratory, endovascular, central nervous system and urinary infections. *S. aureus* is a medically important organism because it is responsible for various infections and emerging antibiotic resistance. *S. aureus* colonization can persist for months to years and remains asymptomatic in many individuals. Production of coagulase enzyme is a virulence factor and diagnostic feature of the organism. A study showed that following nosocomial infection, colonized individuals had less severe *S. aureus* disease compared to non-colonized individuals [19]. *S. aureus* which are resistant to common antibiotics especially to methicillin is called as Methicillin Resistant *Staphylococcus aureus* (MRSA). There are two types of MRSA-Hospital and Community Acquired MRSA.MRSA infections are more virulent and difficult to treat than *S. aureus* infections due to the antibiotic resistance. The epidemiology of MRSA is now changing from hospital setting to healthy community dwelling individuals without established risk factors for the acquisition of MRSA [6]. The CDC definition of CA-MRSA infection includes**:** positive culture for MRSA as an outpatient or within 48 hours of hospital admission, no medical devices or indwelling catheters that are permanently placed through the skin, no history of MRSA infections, no recent history of hospitalization or residence in nursing home or long-term care facility [17]. MRSA causes minor or severe skin infections, surgical wound infections, blood stream infections and pneumonia [12]. MRSA strains harbor the mecA gene in the genomic island of SCC (Staphylococcal Cassette Chromosome) mec, which encodes the low affinity Penicillin binding protein2a (PBP2a). MecA gene stops beta lactam antibiotics from inactivating the transpeptidases which is critical for cell wall synthesis. PBP 2a confers resistance to otherwise inhibitory concentration of all beta lactam antibiotics [6]. There are six types of Sccmec designated as type I – VI. Type I- III Sccmec are larger element isolated from HA-MRSA strains and type IV and V are smaller elements and isolated from CA-MRSA strains [10]. The mecA gene and the regulatory components together comprises mec complex [11]. In addition SCC mec also carries mecR1 and mecI which regulates the expression of mecA, with increased mecA translation induced by beta lactam antibiotic exposure. MRSA spreads by direct skin to skin contact with an active infection or by contact with contaminated fomites and surfaces [12]. Public fitness centers and exercise facilities have been implicated as possible sources for transmitting community acquired bacterial infections [13]. This study mainly aims at identifying the risk of MRSA colonization and contamination in a gymnasium community and determining the antibiotic resistance of community acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA).

2. Literature Survey

The pathogenesis of CA-MRSA and HA-MRSA was described. The results showed anterior nares are the classic reservoir for nosocomial *S. aureus* infections including HA-MRSA. Some MRSA strains contain factors or genetic backgrounds that may enhance their virulence or may enable them to cause particular clinical syndromes [16].The study described about patients with MRSA-positive body sites,

MRSA contamination on their surrounding environmental surfaces and the palm of their dominant hand were detected and quantified [18]. The existence of common genetic markers among 117 CA- MRSA isolates from 3 continents (Europe, Australia, North America) was assessed. Sccmec type IV element and PVL genes were found common to CA-MRSA isolates from all locations. Most strains also harboured the related luk E-luk D genes of another leukocidin frequently recovered from patients with Staphylococcal infections [4]. The detection of MRSA using a new medium BBL CHROM agar MRSA (CMRSA) was demonstrated. CMRSA is a selective and differential medium for The detection of MRSA using a new medium BBL identification of MRSA that produces mauve colored colonies in presence of chromogenic substrate and cefoxitin. Compared to current methods of identification such as oxacillin screen agar, PBP2a latex agglutination test and cefoxitin disk screening test CMRSA produced sensitivity of 82% and specificity of 99.5%. There is increase in sensitivity to 91% after 48 hours. Total time required for identification by CMRSA agar is 24-48 hours whereas other methods requires 48-72 hours [15]. The rapid, qualitative detection of MRSA was showed directly from nasal swab specimens by Real- time PCR assay and direct detection of MRSA by amplicon hybridization with a fluorogenic target specific molecular becon probe. IDI-MRSA (Infectio Diagnostic, Inc., Sainte-Foy, Qu´ebec, Canada) assay was found to provide more sensitivity and specificity [2]. The prevalence and risk factors for CA-MRSA colonization and changes in colonization over the time in US army soldiers were evaluated. The clinical significance of the colonization was determined by swab samples of nares. Demographic data were obtained and the participants were monitored for skin and soft tissue infection. *S. aureus* isolates were characterized by *in vitro* examination of antibiotic susceptibilities, *mec*A confirmation, pulsed-field gel electrophoresis, and Panton-Valentine leukocidin gene testing [9].

3. Materials and Methods

The gymnasium community people were selected for the study of methicillin resistance *S. aureus* (MRSA). A total of 175 samples were collected from 35 men and women who regularly work out in gymnasium between the age group of 20-50 years. From each individuals, samples were collected from the sites of palm, forearm, nose, shoe and towel using sterile cotton swabs. The prior permission from the gymnasium for sample collection and informed written consent (questionnaire type) from the volunteers were obtained for sample collection during the study.

3.1 Transport of Samples

After sample collection, the samples were placed in transport media, labeled and transported to the microbiological laboratory within 1-2 hours.

3.2 Processing of Specimens

The labeled samples were directly inoculated onto sterile mannitol salt agar (Hi media Laboratories, Mumbai) plates and incubated at 37º C for 24 hours. The colonies obtained were differentiated as yellow, pink and white. It was inoculated onto nutrient agar for further testing. Preliminary tests such as Gram's staining and catalase tests were performed. The samples which were catalase positive and Gram positive cocci in clusters were subjected to oxidative fermentation test, mannitol fermentation test and coagulase test. The culture which showed the following characteristics were identified as S. *aureus*

- Yellow colonies on mannitol salt agar.
- Gram positive cocci in clusters.
- Golden yellow colonies on nutrient agar
- Catalase positive.
- Oxidative and fermentative
- Mannitol fermentation with acid production.
- Coagulase (tube test) positive.

3.3 Antibiogram Tests

Antibiogram test using Kirby Bauer method was performed for the isolated *S. aureus* strains using antibiotic discs. For the confirmation of methicillin resistance cefoxitin disk screening test (30 µg) [5] and oxacillin agar screening test (6mg/l) (Acumedia) was performed. Minimum inhibitory concentration- agar dilution method for the resistant strains (Kirby Bauer method) against oxacillin using various concentrations was determined [7].

4. Results

- Out of 175 samples collected from different sites, 56 (32%) have shown golden yellow colonies on mannitol salt agar. Out of 56, 14 were nasal samples (highest).Some samples did not show any growth on mannitol salt agar (chart 1).
- 9 samples were identified as *Staphylococcus aureus* showing characteristic features (coagulase positive) (figure 1) (chart 2).
- The antibiotic susceptibility of the organisms were studied by Kirby Bauer method and zone size for each antibiotic was measured and results were interpreted by comparing the test culture with ATCC *S.aureus* 25923 (figure 2)
- Among the *S. aureus* cultures tested, the number of isolates which showed resistance to each antibiotic were determined. 86% of test cutures showed resistance to Methicillin (30µg), amoxycillin (30µg) and penicillin (10 U), 71% (n = 5) to bacitracin (10 U), 57% (n = 4)of cultures showed resistance to vancomycin (30µg), novobiocin (50 µg) and furazolidone (50 µg) (table 1).
- The methicillin resistance of the organisms was identified using cefoxitin disc (30 µg) and oxacillin agar screening methods. Minimum inhibitory concentration of the methicillin resistant strains positive for Kirby Bauer method against oxacillin was performed and the results were interpreted (figure 3a and 3b)
- Finally 1 resistant MRSA strain was identified using cefoxitin disc and oxacillin agar screening test (6mg/l). Minimum Inhibitory Concentration (MIC) of the MRSA strain against oxacillin by agar dilution method was found to be ≥128mg/l (figure3c) (table3)

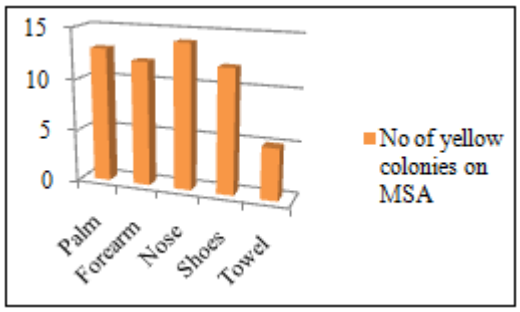

Chart 1 Growth of *Staphylococcus aureus* on mannitol salt agar

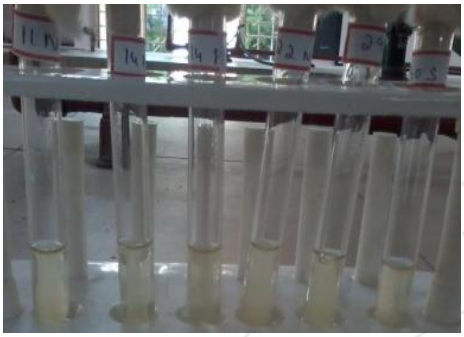

Figure1 Coagulase tube test
Clot formation indicates coagulase positive

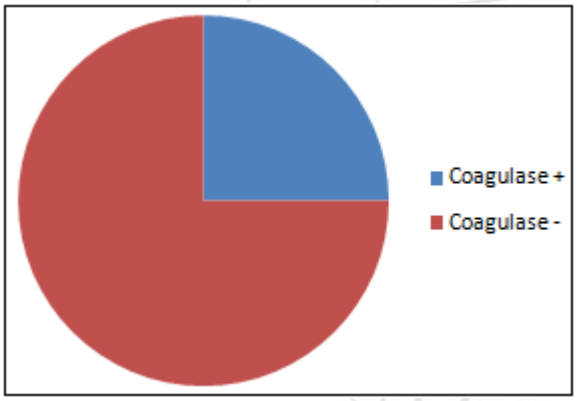

Chart 2 Isolation rate of *S aureus*

Coagulase positive- *S. aureus*- 25%
Coagulase negative- Other species of *Staphylococci*- 75%

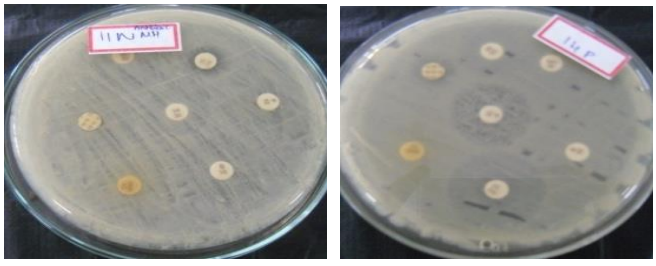

Figure 2 Antibiogram test- Kirby Bauer method
No zone formation indicates resistance to each antibiotic

Table 1: Antibiogram test- Kirby Bauer method

S.NO			*TEST CULTURE*			*ZONE OF DIAMETER (mm)*		
		MET 30	*AMX 10*	*P 10*	*B 10*	*V 30*	*NV 30*	*FR 50*
1	ATCC *S.aureus* 25923	22-32	28-36	26-37	12-22	17-21	22-31	18-22
2	11N	-	-	-	-	-	-	-
3	14P	-	-	21 R	-	-	27 S	-
4	$14NC_1$	25 S	32 S	25 R	20 S	24 S	30 S	25 S
5	22N	-	-	18 R	15 S	19 S	20 R	18 S
6	26T	-	-	-	-	8 R	16 R	18 S
7	29F	-	-	-	-	-	-	-
8	30S	-	-	30 S	-	20 S	25 S	-

Test culture:
P: palm, N: nose, NC1: nose colony 1, T: towel, F: forearm, S: shoe
Zone of Diameter:
R = Resistant, S = Sensitive, - = No zone formation (resistant)
MET 30- methicillin 30 µg, AMX 10- amoxycillin, 10 µg
P 10- penicillin 10 U, B 10- bacitracin 10 U, V 30- vancomycin 30µg, NV 30- novobiocin 30 µg and FR 50- furazolidone 50 µg

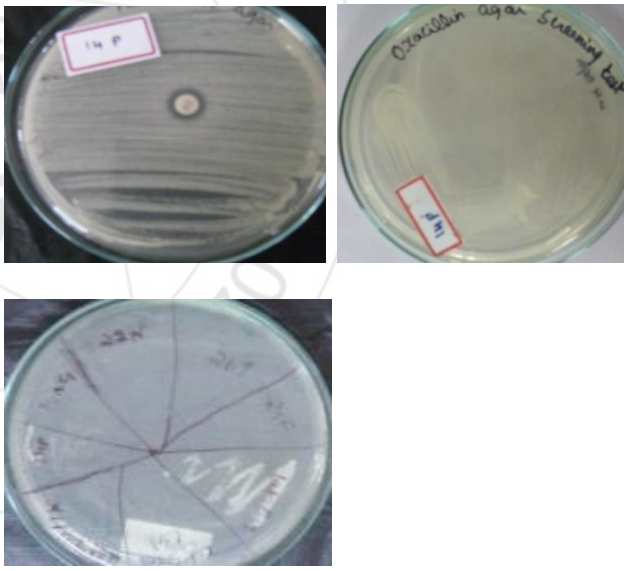

Figure 3a, 3b and 3c Screening tests

a. Cefoxitin disc test
b. Oxacillin agar screening test (6mg/l)
c. MIC- Agar dilution method (128 mg/l)

a. Zone size ≤ 21mm around cefoxitin disc indicates methicillin resistant strain

b. Growth in oxacillin agar indicates methicillin resistant strain

c. Growth in oxacillin concentration of 128 mg/l indicates methicillin resistant strain

Table 2: Isolation rate of *S. aureus* and MRSA

S. no	*Name Of The Test*	*Total Isolates (N)*	*Postive S. aureus Isolates*	*Percentage Of S. aureus*	*Percentage of MRSA*
1	Coagulase	36	9	25 %	-
2	Oxacillin agar screening (6mg/l)	6	1	100%	17%

Table 3: Minimum inhibitory concentration against oxacilin- Agar dilution method

S. No	*Test Culture*	*Concentration (mg/l)*										*MIC (mg/l)*
		128	64	32	16	8	4	2	1	0.5	0.25	
1	11N	-	-	-	-	-	-	+	+	+	+	4
2	14P	+	+	+	+	+	+	+	+	+	+	-
3	$14NC_1$	-	-	-	-	-	-	+	+	+	+	4
4	22N	-	-	-	-	-	-	+	+	+	+	4
5	26T	-	-	-	-	-	-	+	+	+	+	2
6	29F	-	-	-	-	-	-	+	+	+	+	2

+ = Growth , - = No growth

Table 4: Cumulative results of screening tests

S. NO	*Name of the Screening Test*	*Total Isolates*	*Positive S. aureus Isolates*	*Percentage of MRSA*
1	Kirby-Bauer method	7	6	71
2	Cefoxitin disk screening (30 µg)	6	1	17
3	Oxacillin agar test (6mg/l)	6	1	17
4	MIC agar dilution	6	1	17

5. Discussion

The purpose of the study was to detect MRSA in gymnasium community. A total of 175 samples were collected from 35 men and women of gymnasium community. The samples were collected from the sites of palms, forearms, noses, shoes and towels and inoculated on MSA. In a study, a total of 310 swabs were collected from wounds, hands and doctor's mobile phone to isolate MRSA pathogens [1]. This study isolated 32% (n = 56) golden yellow colonies on mannitol salt agar which is similar to previous study where they 35% from community [21]. Out of 56 samples, only 25% (n = 9) were coagulase positive which was relatively low compared to the results of similar study [21]. Out of 9 isolates obtained 7 was known to be resistant to mehicillin by Kirby Bauer method. All the 7 MRSA isolates were subjected to cefoxitin disc test, oxacillin agar screening test and MIC agar dilution test. All the three study showed only 17% of positive MRSA isolates which was found to be similar to the research study where the MRSA prevalence was 22% isolated from staffs, fomites and students of Faculty of pharamaceutical sciences [20]. The study showed the importance of disinfection or handwashing before and after exposure to gymnasium since there is a risk factor for MRSA contamination. The unhygienic hand swab showed the presence of MRSA and improvement in hand hygiene showed reduction of nosocomial infection and MRSA transmission [3].

6. Conclusion

We conclude the study as follows: Cefoxitin test (30µg) can be used for the determination of mecA gene which mediate methicillin resistance in *S. aureus* strains since oxacillin screening test and polymerase chain reaction are expensive. This test can be a simple preliminary test for qualitative detection of MRSA. Initially screening of samples from nose, palm, shoes and towel revealed the highest number of yellow colonies from nasal samples but revealed final resistant strain (n = 1) from palm sample which was not considered very high but shows the prevalence or contamination of CA-MRSA in gymnasium community. The study can be extended by molecular characterization of mecA gene by polymerase chain reaction which is the gold standard method for MRSA detection.

7. Future Scope

Identification of CA-MRSA will be more quick, reliable and appropriate when the strain is treated for mecA gene using polymerase chain reaction. After the confirmation of mecA genome (gold standard method). The volunteers who carry the MRSA strain should be treated with appropriate antibiotics or sanitation methods in a community to control the further spread of CA-MRSA. If the asymptomatic carriers of MRSA in a community were not treated at right time, the resistant strains will infect them when the immune system is weakened and the infection spreads rapidly within and outside the community. Hence the future studies of this research can be extended by genome sequencing of the isolated strain and appropriate treatment of potential resistant MRSA carrier.

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