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Detection of MRSA Carriers among Health-Care Workers and Patients In A Tertiary Care Hospital As An Active Surveillance Measure.

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ABSTRACT

Staphylococci, especially Methicillin-resistant Staphylococcus aureus (MRSA) isolates became one of the potent nosocomial pathogen to cause significant mortality and morbidity. Individuals colonized by MRSA acts as reservoir and also disseminate infections to others. Especially in case of intensive-care patients, MRSA worsens their morbidity and increases the mortality and also creates financial burden. Thus this study was aimed to detect the percentage of MRSA carriers in various anatomical sites among in-patients, out-patients and in health-care workers A total of 900 swabs were taken from all the three groups of study population from different anatomical sites. Samples were inoculated on to Mannitol screening agar and *Staphylococcus aureus* isolates were identified. MRSA isolates were confirmed by various phenotypic methods like Cefoxitin disc diffusion method, by inoculating onto Oxacillin and vancomycin screening agars. Mupirocin susceptibility and Vancomycin MIC were confirmed by appropriate methods. Total of 14 MRSA carries were identified out of 900 swabs(450 individuals). MRSA carrier rate was identified as 3.1%(14/450) in our tertiary care setting. In-patient occupies the highest of 5.3% and followed by Health-care workers 4%. Eighty eight percent of in-patients MRSA carriers and 10% of Health-care MRSA carriers were identified by anterior nares sampling. Mupirocin susceptibility for all 14 MRSA isolates by Kirby Bauer disc diffusion method showed 100% susceptibility. Vancomycin MIC by agar dilution and E-test methods showed MIC value of ≤1µg/mL. Though it is always controversial whether to screen for MRSA carriers among health-care professionals and others, we have done this study as an active tool to prevent our hospital acquired infections due to MRSA. Among in-patients regular screening and decolonization will strongly bring down the morbidity and mortality. Anterior nares are found to be ideal site to identify MRSA carriers among our study population.

Keywords: MRSA carriers, Healthcare workers, outpatients, MRSA, Vancomycin, Nasal screening.

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INTRODUCTION

The development of antimicrobial resistance among nosocomial pathogen creates a serious threat to public health. Methicillin resistant *Staphylococcus aureus* (MRSA) is one among the potent nosocomial pathogen leading to significant increase in morbidity and mortality worldwide [1]. MRSA are those *Staphylococcal* isolates, which are resistant to penicillinase stable penicillins like methicillin, oxacillin, dicloxacillin and flucloxacillin. This resistance is mediated by the presence of a novel penicillin binding protein (PBP2a) [2].

Diseases caused by MRSA may vary from mild to fatal, which includes localized infections like folliculitis, furuncles, abscesses, carbuncles, paronychia and invasive infections like endocarditis and septicemia [3]. Patients once colonized with MRSA, are of increased risk 11-25% to develop acute and 3-15% chronic infections in their subsequent one year when compared to non-colonized healthy individuals [4-7]. Colonized individuals serves as reservoirs of self-infection or dissemination to other patients and to the hospital environment (Colonization is the presence of non-pathogenic microorganisms on the host)[8]. Asymptomatically colonized patients and Health care workers (HCWs) are the major sources of MRSA in the hospital environment [9]. Most of the time it is transmitted through the hands of health-care workers rather than *de novo* [10]. Hospital beds, bedpans, stethoscopes, patients care delivering systems like, ventilator tubing's, intravascular devices and urinary catheters also acts as other sources [11]. MRSA infection is a significant contributor for prolonged hospital stay and increase in hospital expenditure amongst surgical and intensive care patients and also responsible for poor clinical outcomes.

In the recent past, MRSA is not only prevalent in the health-care settings, due to some associated factors like, prior antibiotic usage, contact with health care facility providers, poor socio-economic conditions and overcrowding, it is found to be prevalent in community also [12,13]. Thus this is aimed to know the proportion of MRSA carriers among the patients (both out-patients and in-patients) and HCWs, to detect the Mupirocin susceptibility by disc diffusion method, to detect the Vancomycin MIC for all identified MRSA isolates. Finally we hope our active surveillance study will provide a good data for the policy makers in our region to know the current MRSA status.

MATERIALS AND METHODS

A prospective analytical study was conducted for two months during July and August 2014 in a tertiary care hospital Puducherry, India, involving three groups with a total of 450 population (900 swabs). Outpatients- 150, In-patients – 150, Health-care workers- 50, residents 50 and consultants- 50(150). Institute ethical committee clearance was obtained.

Sample collection

Each two sterile disposable swabs were used for screening all the subjects. Swabs were taken from the following sites, in case of

- In-patients: One from anterior nares and another one from axilla.
- Out-patients: One from anterior nares and another one from throat.
- Consultants: One from anterior nares and another one from palms and web spaces [13, 14].

Inclusion criteria

- In-patients - patients admitted in critical-care units, dialysis unit, post-operative ward, OBG ward, surgery ward and orthopedics ward.
- Out-patients – patients who have not visited any health-care providing setup and not contacted with any of the health care workers for the past 1 year period.
- All HCWs associated with patients services – Nurses and paramedical staffs.
- Consultants includes – doctors, post graduates and interns.

Exclusion criteria

- Patient with known MRSA infection,
- Out –patients with previous history of hospitalization within a year of time, with chronic illness
- Age below 15 years of age.

Swab samples from various anatomical sites were immediately transported to the Microbiology laboratory and were inoculated onto Mannitol salt agar (MSA) and incubated at 37°C ambient air for 24 – 48 hrs. Colonies suggestive of *S.aureus* (yellow colonies) (fig. 1) were further confirmed by Gram's staining, catalase, slide and tube Coagulase tests and urease test. MRSA isolates were confirmed by Kirby Bauer disc diffusion method using Cefoxitin (30µg) disc (fig.2) and incubated at 33-35°C, ambient air for 16-18hrs. Zone of inhibition measuring ≤21mm were considered as MRSA isolates. Further all isolates were confirmed by spot inoculation onto Oxacillin screening agar (MHA with 4% Nacl and 6µg/mL of Oxacillin).[2,15] (Fig. 3)

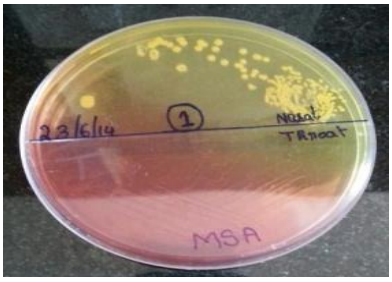

Figure 1: Mannitol Salt Agar (MSA) showing yellow colonies

Figure 2: Cefoxitin disc diffusion test showing *ATCC S.aureus, ATCC MRSA* and one of the test MRSA positive strains.

Figure 3: Oxacillin screen agar (OSA) test showing grids with ATCC control strains and 14 tube coagulase positive strains

Figure 4: Mupirocin susceptibility testing by Kirby-Bauer disc diffusion method

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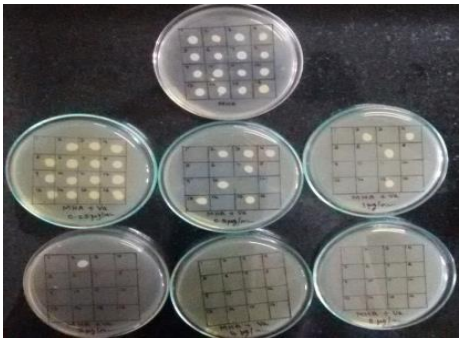

Figure 5: Vancomycin MIC by Agar Dilution method with *ATCC S.aureus* and *ATCC MRSA* all 14 MRSA isolates

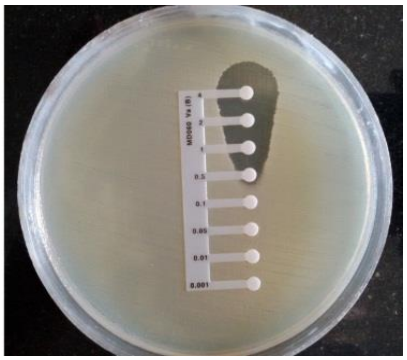

Figure 6: Vancomycin MIC for E test technique.

For all MRSA isolates Mupirocin susceptibility by disc diffusion method was made (fig.4). Vancomycin solution with concentration ranging from 16µg/mL to 0.25 µg/mL was prepared and MIC was checked by agar dilution (fig.5) and further confirmed by E test methods (fig.6). Strains of MRSA (ATCC 43300) and *Staphylococcus aureus* (ATCC 25923) were used for quality control for all the mentioned procedures.
The MIC interpretation range for vancomycin as follows [2,15].

- Sensitive ≤2µg/mL, Intermediate -4-8µg/mL & Resistant ≥16µg/Ml

Methods of statistical analysis

Proportion, 95% confidence interval, chi-square test will be used for analysis.

RESULTS

A total of 450 individuals were screened for the presence of MRSA by taking 900 swabs from various anatomical sites.(Table 1) Out of this 450 individuals, among 150 out-patient who attended our tertiary care hospital for various clinical consultation during the study period for the first time, 54%(81) were males and 46%(69) were females. And the majority were between the age group of 18 and 35 years of age.(Table 2) None of the out-patients among the study population (0%) were found to be MRSA carriers from their nasal and throat swabs (150 nasal swabs & 150 throat swabs).

Table 1: Distribution of total swabs taken from various sites

Sl. No	Distribution of total study subjects	Total number of swabs taken =900 swabs			
		Anterior nares	Axillary swab	Web spaces	Throat swab
1	Out-patients	150	0	0	150
2	In-patients	150	150	0	0
3	Health-care workers	150	0	150	0
	TOTAL (900 swabs)	450 swabs	150 swabs	150 swabs	150 swabs

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Table 2: Age-wise distribution of out-patients

Age-wise distribution of Out-patients	
Age distribution	Total no. of cases
18-35 years	71(47%)
36-50 years	57(38%)
51-65 years	14(9%)
>66 years	8(5%)

Among 150 in-patients who got admitted to critical-care units(MICU, ICU, CCU), dialysis unit and Post-operative wards (surgery, orthopedics, obstetrics & gynecology), 77%(116) were male and 23%(34) were female. According to age distribution, the majority of screened patients were between the age of 51 and 65years (Table.3).

Table.3: Age-wise distribution of In-patients :

Age-wise distribution of In-patients	
Age distribution	Total no. of cases
18-35 years	35(23%)
36-50 years	32(21%)
51-65 years	53(35%)
>66 years	30(20%)

Among 150 health-care worker populations (includes – Consultants, senior residents, junior residents, interns, nursing staffs and other health-care facility providing Para-medical staffs), majority 63%(95/150) were female and 37%(55/150) were males.

A total of 14 MRSA strains were isolated from all 450 study subjects (900 swabs), giving an overall MRSA carrier positivity rate of 3.1 % (14/450). Eight of them were from in-patients (8/150=5.3%) and remaining 6 were from health-care workers (6/150=4%). None of the 150 out-patient swabs either nasal or throat swab showed presence of MRSA. Thus from the community 0% positivity was noticed [out-patients study subjects (0/150)]. (table 4). Majority of the identified MRSA carriers were males (11/132, 8.3%) and 2.3% were females (3/129,2.3%)(Graph 1). Among 8 in-patients MRSA carriers, patients from critical care units(MICU 3 patients and ICU 3 patients) which accounted for the highest MRSA carrier rate of 75%(6/8). The remaining 2 MRSA carriers were from, orthopedic surgery ward 1(12.5%) and from general surgery ward 1(12.5%) patient respectively. Seven out of 8 carriers (88%) were found to be colonized in their anterior nares, of which 4(50%) of them were with MRSA in their anterior nares alone and remaining 3(37.5%) of them were found to carry the organism in their anterior nares and also in their axilla also. Whereas only one (1/8,12.5%) of them was found to carry MRSA in their axilla alone.(table 4). Out of 150 in-patients, 7(4.7%) were identified by anterior nasal swab and 4(2.7%) were identified by axillary swab. The difference in proportion was tested and found statistically not significant (P value=0.36).

Table 4: Distribution of MRSA colonizers identified

Total number of MRSA isolated = 14 study subjects					
Sl.no.		Anterior nares alone	Anterior nares & Axilla	Axilla	Throat/Web spaces
1	Out-patients	0	0	0	0
2	In-patients	4	3	1	0
3	Health-care workers	6	0	0	0
TOTAL		10	3	1	0

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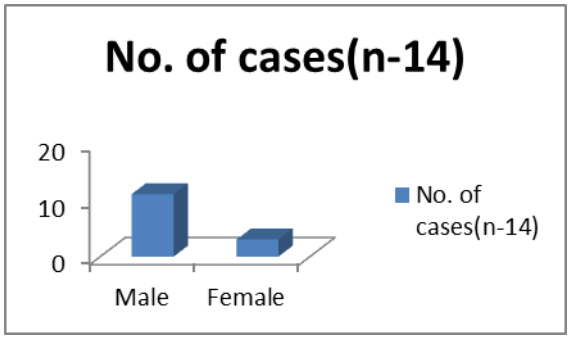
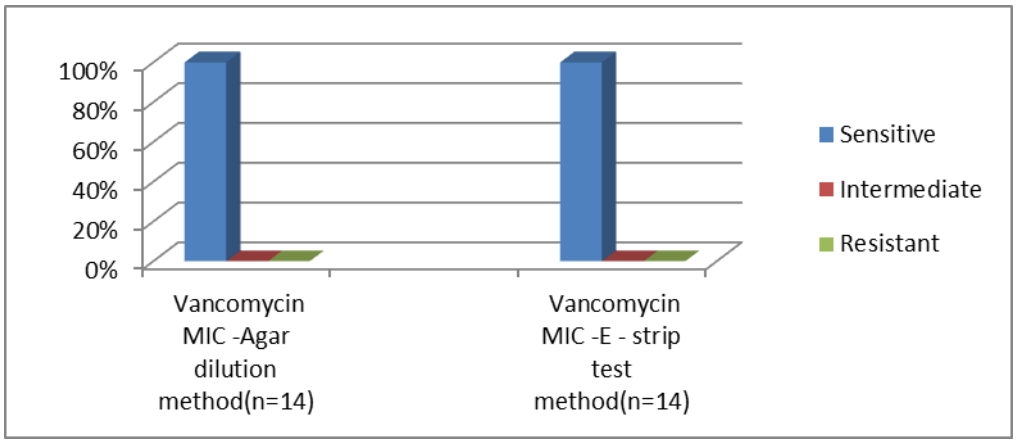


Graph 1: Sex-wise distribution of identified MRSA positive colonizers.

Out of these 150 health-care workers, a total of 6(4%) nasal MRSA carriers got identified from their nasal swabs. Among these 4% nasal MRSA carriers, senior residents occupies the highest of 50% (3/6), followed by them, 33%(2/6) were staff nurses and finally 17%(1/6) were Para-medical workers. Among this 150 health-care workers, 6(100%) MRSA carriers were detected by nasal swabs and none of them were detected by using web space swabs. The difference in proportion was tested and found statistically significant (P value= 0.014).

Mupirocin susceptibility for all 14 MRSA isolates by Kirby Bauer disc diffusion method showed 100% susceptibility. Vancomycin MIC by agar dilution method for all MRSA isolates showed MIC value of ≤1µg/mL (within sensitive range). Controls satisfactory (*ATCC S.aureus)*. Further confirmation by vancomycin E-test also showed satisfactory sensitive value of ≤1µg/mL for all isolated MRSA isolates.(Graph 2)



Graph 2: Vancomycin MIC by agar dilution and E-strip method.

DISCUSSION

Antimicrobial resistance is one of the major universal health issues. Prevalence of resistance pathogens, including community and hospital acquired MRSA have become more prevalent. Following the first report of MRSA infection reported in United States at the Boston city hospital during 1961, this pathogen emerged worldwide. Active surveillance measures like detection of MRSA infection and colonization among patients and health-care workers will reduce the burden of nosocomial spread [15].

The prevalence of MRSA varies considerably among various countries ranging from <1% in Sweden, Norway and Netherlands, Austria 8.2%, 11.5% in Switzerland , Germany 19.5%, France 24.5%, Italy 33.5% to >50% in Portugal [3]. In India, prevalence of MRSA is of 30-50% of all *S.aureus* clinical isolates [16]. High prevalence of MRSA got reported earlier ranged between 34.8 and 70.6% [17,18]. Very recent studies represents that MRSA is more endemic in our country, with the estimated rate of 27%, 49% and 47% amongst clinical isolates of *S.aureus* from out-patient, in-patients and ICU patients.[19] A study by Ray et al in 2011, suggested that MRSA carriage rates are nearly 15.6% in in-patients, 3.8% in out-patients and 1.8-25% amongst health-care workers from India [15]. Stephanie Fraser et al., with 7145 surgical in-patients reported 1.88%

MRSA colonization.[20] Our study shows the MRSA positivity rate 3.1%(14/450). Out of this, in-patients 5.3%(8/150), health-care workers 4%(6/150) which is contrast to the above mentioned studies. Other few studies have reported the rate of 6-50% MRSA carriage from health-care workers in burns units [21,22]. Mathanraj et al from Pondicherry, Indian reported the overall positivity rate of 8.5% MRSA carriers. Out of this, 15.6% were from inpatients, 3.8% were outpatients and 1.8% were belong to health-care workers which is closely co-relating with our study [13]. According to Prentice et al., the proportion of MRSA ranged from 4% to 8% [23].

A meta-analysis study regarding prevalence of CA-MRSA with 50,737 patients from 33 studies by Shipeng Li et al., showed 39% CA-MRSA prevalence.[24] Debora A Tavares et al., with 2,100 samples only <1% CA-MRSA was documented [25]. We reported 0% MRSA from out-patients which is not similar to other studies.

Fraser et al., reported 48.5% were females and 51.5% were males.[20] Mathanraj et al., of which the majority overall were of males (12.4%) when compared to females of 2.4%.[13] Fomda et al reported 1.83% of MRSA colonization from nasal swabs. Among these, 53% were males and 46% were females [26] we reported 11(79%) of MRSA carriers from male population and 3(21%) were of females which is similar to other studies. Considering financial expenditure during screening programs, selections of appropriate anatomical sites need to be more focused.

A study conducted by Nagarajan et al in Chennai, South India, reported the overall nasal carriage of MRSA was 3.7% [27]. Several studies have showed that nasal carriage of *S.aureus* plays an important role in both hospital and community acquired MRSA infections [28]. Study by Khalid El Bouri et al., with seven different human anatomical sites from 4,769 individuals, reported 19.4% MRSA positive incidence. According to their result, out this MRSA cases, 50.5% were isolated from nasal region swab alone(single site). Which is more similar to our study(50%). To improve the MRSA detection rate more than one anatomical sites swabs will be effective when compared to single nasal swab [29]. With 21 various possible anatomical site combination, they reported groin and throat (74.5% detection) combination was the best when compared with others, followed by others like nose and throat etc. According to them, nose +throat and nose+axilla combination identified 71.6% and 56.6% of MRSA colonizers respectively [20]. In addition to the above mentioned, we have included nose+web- spaces/palms. Our study, we reported 50% MRSA carriers through anterior nares swabs alone. From axillary swab and anterior nares (multiple site) combination 37.5% and from axillary swab alone 12.5% MRSA cases got identified(single site). Which is closely similar to others. Vinodhkumaradithyaa et al, reported 15.4% MRSA nasal carriers, of this 30.6% were from staff working in one particular surgical ward [30]. All the MRSA isolates identified from health-care workers in our study is from their nasal swabs only, thus our results are well co-relating with others study.

As Oxacillin is not reliable, cefoxitin(30µg) disc is used as a surrogate for oxacillin, thus oxacillin susceptibility must be reported based on cefoxitin disc zone interpretation only according to CLSI 2013 guidelines(zone ≥22mm sensitive, ≤21mm resistant). We also followed the same criteria for identifying all MRSA isolates.

Regarding mupirocin susceptibility testing, although not formally validated by CLSI document M23-based analyses, some studies have linked a lack of response to mupirocin- based decolonization regimens with isolates for which the mupirocin MIC are ≥512µg/mL. CLSI has given interpretation to assess high-level mupirocin resistance for *S.aureus* only i.e. any zone- represents absence of high-level mupirocin resistance. Considering these criteria, all our 14 MRSA isolates showed a very wide zone of inhibition around the mupirocin disc, representing all isolates will respond to mupirocin decolonization regimens. Thus the spread can be prevented. Prospective studies suggested that, the application of Mupirocin calcium hydrate ointment to the nasal cavity and the use of appropriate pre-operative antibiotics reduce the incidence of MRSA infections among high risk patients [20]. All our identified MRSA harboring health-care workers were given alterative employment and underwent decolonization program with ethical issues consideration and by maintaining strict confidentiality. Resampling was made and advised to continue their health service as before. But in case of in-patients decolonization schedule could not be completed, considering various patients factors.

For reporting vancomycin susceptibility ideally MIC testing should be performed to all the isolates of *S.aureus*. The vancomycin disc test does not differentiate vancomycin susceptibility from vancomycin intermediate isolates of *S.aureus*. Our all 14 MRSA isolates showed MIC range within susceptible limits (Sensitive-≤ 2µg/mL, intermediate-4-8µg/mL, resistant ≥16µg/mL) by agar dilution method. We further confirmed our results with Hi-media vancomycin E-stripes also, it also showed the same sensitive limits of MIC only. A very recent study done by Chaudhari et al with 232 clinical MRSA isolates, showed 93.5% susceptible MIC value by both agar and E test methods [31] which is similar to our study results.

CONCLUSION

Though many studies have reported marked prevalence of community acquired MRSA, fortunately we have not identified any CA-MRSA. Among in-patient population, majority of MRSA isolated were from critical care units only, thus in those area active long-term surveillance studies need to be planned in future. In-case of health-care workers, resident population acts as a good reservoir for harboring MRSA colonization. Thus periodic MRSA screening programs, identification of all sources, source isolation and decolonization need to be followed among all health-care professionals and in-patients in all health-care setting though it is always controversial.

We hope that our study will help the health-care professionals to decide the appropriate strategy need to be adopted for optimizing the MRSA colonization detection, and to bring down the nosocomial infection and its spread among patients and health-care workers. However, the burden of nosocomial pathogens can be managed and reduced by strictly adopting various components of Routine Practices like: hand hygiene, use of personal protective equipment (PPE), accommodation, patient care equipment, the general environment and patient transport.

Limitations

Guidelines says that, if screening result is positive, a decolonization regimen should be commenced as soon as possible irrespective of the availability of patientsisolation facilities, to reduce the number of microbes present on an infected individuals.[32] Considering the ethical issues we couldn't do so in all MRSA positive cases. Sample size is not very significant as we have not covered the whole hospital population. Molecular method of confirmatory test, is not done due to lack of opportunity. Multicentric study is needed to measure the accurate prevalence of MRSA among these three groups of study population for long term in the future.

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