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Prevalence of Methicillin Resistant *Staphylococcus aureus* and Minimum Inhibitory Concentration (MIC) of Vancomycin Against it in Two Selected Semi Urban Sri Lankan Communities

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Abstract: ***Background: Methicillin Resistant Staphylococcus aureus (MRSA) infection has become a major problem within the hospitals and in the community. The aim of this study was to describe the prevalence of MRSA nasal colonization in the community, antibiotic sensitivity patterns of these isolates and MICs of vancomycin. Methods: Nasal samples for Staphylococcus aureus culture and sociodemographic data were obtained from adults and children ≥2 years of age from the two communities. Staphylococcus aureus isolates were identified by routine laboratory methods and antimicrobial susceptibility tests were done by using the CLSI guidelines. VancomycinMICs were tested for both MRSA and Methicillin Sensitive Staphylococcus aureus (MSSA) isolates. Results: A total of 317 subjects, 88 (28%) were positive for Staphylococcus aureus. Of the 88 isolates, 18 (5.7%) were MRSA and 70 (22%) were MSSA. Of the MRSA isolates the high sensitivity noted in Linezolid, Ciprofloxacin, Trimethoprim-Sulfamethoxazole, Tetracycline Rifampin and Gentamicin. Inducible Clindamycin resistance was reported 44%, 7% for MRSA and MSSA isolates respectively. Vancomycin MICsof all the isolates were ≤ 2µg/mL. Conclusions: MRSA isolates of this study are more likely to be community acquired. However, further molecular studies are needed to confirm these findings.Both MRSA and MSSA isolates vancomycin MICs were≤ 2µg/mL.However, MRSA strains showed higher level vancomycin MIC compared to MSSA.***

Keywords: Methicillin resistant Staphylococcus aureus, community acquired, Minimum inhibitory concentration

1. Introduction

Staphylococcus aureus is a frequent cause of serious infections but also a common human commensal. It can be found on skin,nose, axilla and groin.[1]Damage to the skin or other injury may allow the *Staphylococcus aureus* to overcome the natural protective mechanisms of the body, leading to an infection. It causes many superficial and deep infections including skin and soft tissue infections, pneumonia, septic arthritis, osteomyelitis, foreign body infections and sepsis.[1] Emergence of multiple resistances to anti-microbial agents has made treatment of staphylococcal infections difficult. The resistance to beta lactam antibiotics is mainly indicated by resistance to methicillin. Therefore, these bacteria are known as Methicillin Resistant *Staphylococcus aureus* or MRSA. Currently, MRSA is a serious health concern.[2]There are two types of MRSA. health care associated (HA-MRSA) and community associated (CA-MRSA). Health care associated strains can be found in hospitalized patients and other health facilities. These strains are resistant to other groups of antibiotics as well. CA-MRSA strains can be found in the community.CA-MRSA are generally sensitive to other groups of antibiotics apart from beta lactams.[1]

Many studies show that, prevalence of CA-MRSA is increasing.[3]Therefore, not only HA-MRSA, CA-MRSA is also a growing threat to the general public.[4] Common risk factors for the MRSA infections include prolonged hospital stay and the frequent use of antibiotics. Community acquired strains have been distinguished from their HA-MRSA counterparts by molecular methods. HA-MRSA strains carry a relatively large staphylococcal chromosomal cassette mec or SCC mec belonging to type I, II or III. They are often resistant to many classes of non-β lactam antimicrobials.[1] Incontrast, CA-MRSA isolates carry smaller SCC mec elements, most commonly SCC mec type IV or V. They are resistant to few non-β lactam classes of antimicrobials and frequently carry Panton Valentine Leucocidin or PVL gene. In addition to these genotypic characteristics, CA-MRSA infections tend to occur in healthy young children and in elderly and predominately associate with skin and soft tissue infections, which can spread rapidly.[1]

Vancomycin usage has been increased due to increased incidence of MRSA infections.[5]The widespread use of vancomycin may result in emergence of MRSA isolates with reduced susceptibility to vancomycin.[6]There is some evidence that the minimum inhibitory concentration(MIC) of vancomycin against *Staphylococcus aureus* isolates to be increasing rapidly, a process referred to as MIC creep.[7, 8] Disc diffusion testing cannot determine the MIC level of vancomycin. MICs are used by diagnostic laboratories mainly to confirm resistance. Our aims were to describe the prevalence of *Staphylococcus aureus* in two selected communities, to assess prevalence of MRSA, to study antibiotic susceptibility patterns, to identify any associated risk factors for MRSA colonization and to study MIC of vancomycin of these isolates.

2. Method

This study was a community based cross sectional, descriptive study. Three hundred and seventeen adults and children over the age of two years in two selected semi urban communities inRatmalana, Sri Lanka, were studied.

S. aureus screening was done for all participants by obtaining a nasal swab and a questionnaire for each household was filled before obtaining the samples.The questionnaire included the socio-demographic data and known risk factors of MRSA carriage including age, gender, previous antibiotic use, previous hospital admissions, residence with a patient of chronic diseases and residence with a person who had been admitted to the hospital. Culture swabs were plated on MacConkey agar and incubated overnight at 35°C. Colonies with distinctive morphology of *S.aureus* were identified by routine laboratory methods and antimicrobial susceptibility tests were done by following the Clinical and Laboratory Standards Institute (CLSI) guidelines.[9]Colonies which were gram positive cocci, catalase positive, coagulase positive and DNAse positive were identified as *S. aureus*. Isolates were screened for sensitivity to cefoxitin (30µg) to identify MRSA.Zone diameters were measured and recorded after 24 hours of incubation at 35°C.Isolates determined to be resistant to cefoxitin were identified as MRSA and sensitive to cefoxitin were identified as MSSA. Inducible Clindamycin resistance andbroth microdilution method for vancomycin were done to determine MIC according to the CLSI guidelines.[10]

3. Statistical Analysis

Data processing and analysis were done by using the Statistical Package of Social Sciences software (SPSS) version 15.

Ethical issues

Approval from the Ethical Review Committee of the Faculty of Medicine, General Sir John Kotelawala Defence University was obtained before the commencement of the study. Permissions to conduct this study were obtained from the Provincial Director of Health Services-Western province, Regional Director of Health Services – Colombo district and the Medical Officer of Health- Dehiwela area.Written consent was obtained from all the participants prior to the study.

4. Results

Population characteristics. A total of 317 subjects were studied (mean age31±19.2), 63% were females. The age group of 2-20 years included 35% of the study population.

***S. aureus* carriage.** 88 were colonized with *S. aureus*. Out of 88 isolates, 18 were MRSA and 70 were MSSA. Low percentages were foundamong MRSA and MSSAcolonized subjects with risk factors such as recent hospitalization, recent outpatient visits, chronic illness, recent antibiotic use and contact with the patients. There were no statistically significant associations between risk factors and MRSA carriage in this study. The socio-demographic data such as age, gender, education level, number of households, monthly family income did not show statistically significant association among MRSA carriage.

Antibiotic susceptibility test.

Disc Diffusion method. All the antibiotics tested, except erythromycinshowed high levels of sensitivityin more than 72% of the cases among MSSA isolates (Table 1). Low sensitivity patterns of erythromycin and clindamycin among MRSA isolates were reported. Other antibiotics showed high level of sensitivity, though these isolates were MRSA (Table 2).

Inducible Clindamycin resistance. Out of 317 subjects, inducible Clindamycin resistance was seenin 7% of MSSA isolates while 44% were resistant among MRSA isolates.

MICs of vancomycin. 50% of MRSA isolatesand 95% of MSSA isolates had MIC value of ≤1µg/mL(Table 3).

Table 1: Antibiotic susceptibilities among MSSA isolates (n=70)

Antibiotic	No. (%) of samples, by susceptibility		
	Susceptible	Resistant	Intermediate
*Cloxacillin	70 (100)		
Erythromycin	31 (44)	19 (27)	20 (28.6)
Clindamycin	55 (79)	5 (7)	10 (14.3)
Trimethoprim-sulfamethoxazole	67 (96)		3 (4.3)
Ciprofloxacin	51 (73)	9 (13)	10 (14.3)
Tetracycline	59 (84)	8 (44)	3 (4.3)
Gentamicin	57 (81)	6 (9)	7 (10)
Linezolid	70 (100)		
Rifampicin	70 (100)		

*Sensitivity tested againstCefoxitin (30µg)

Table 2: Antibiotic susceptibilities among MRSA isolates (n=18)

Antibiotic	No. (%) of samples, by susceptibility		
	Susceptible	Resistant	Intermediate
*Cloxacillin		18 (100)	
Erythromycin	1 (5.6)	13 (72)	4 (22)
Clindamycin	6 (33)	10 (56)	2 (11)
Trimethoprim-sulfamethoxazole	17 (94)	1 (5.6)	
Ciprofloxacin	17 (94)	1 (5.6)	
Tetracycline	14 (78)	4 (22)	
Gentamicin	17 (94)		1 (5.6)
Linezolid	18 (100)		
Rifampicin	18 (100)		

*Sensitivity tested againstCefoxitin (30µg)

Table 3: Vancomycin MICs of MSSA isolates (n=70) andMRSA isolates (n=18)

MIC range (µg/mL)	No. of isolates (%) in MSSA	No. of isolates (%) in MRSA
≤ 0.25	02 (2.9)	
≤ 0.5	13 (18.5)	03 (16.7)
≤ 1	50 (71)	06 (33.3)
≤ 2	05 (7)	09 (50)

5. Discussion

28% of the participants were colonized with *S. aureus* in this study. MRSA prevalence in this study was 5.7%. Similar finding was noted in the studies of Indian communities.[11]Known risk factors for HA-MRSA such as hospital admission, residence with a patient with chronic disease and residence with a patient who was recently admitted to the hospital had no significant association with MRSA carriage in this study. For MSSA isolates, most of

the antibiotics tested were sensitive except for erythromycin which showed only 44% sensitivity. Among MRSA isolates, most of the antibiotics tested were sensitive, except again for erythromycin and clindamycin. These antibiotic sensitivity patterns suggest, all the MRSA isolates were likely to be community acquired MRSA. However, further molecular studies are needed to confirm these findings. According to these results use of erythromycin in empiric treatment of staphylococcal infections may not be useful for children.

In this study, the MICs for vancomycinwere ≤2µg/mL in all MRSA and MSSA isolates. Therefore, all the isolates were in the sensitive range. 50% of MRSA strain showed a MIC of ≤1µg/mL and 93% of MSSA strain showed a MIC ≤1µg/mL. These data suggest that future surveillance for vancomycin MIC should be undertaken to predict the vancomycin efficacy in the treatment of MRSA infections in Sri Lanka.

6. Limitations

All the household members of the selected communities were not included in the study.

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