Nasal carrier of Methicillin resistant Staphylococcus aureus among HIV positive and HIV negative individuals visiting Sukra Raj Tropical and Infectious Disease Hospital, Teku, Kathmandu

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Abstract

Background: MRSA is now endemic, and even epidemic, in many hospitals, long-term care facilities and communities. Moreover, Methicillin resistant strains of *S. aureus*, usually being resistant to several antibiotics, are now presenting the major threat in many different countries throughout the world.

Objectives: To study the prevalence of nasal carriage of *S. aureus* among the HIV positive and HIV Negative individuals and to determine MRSA and VRSA antibiotic sensitivity pattern.

Methods: A cross-sectional study was carried out at Sukraraj Tropical and Infectious Disease Hospital. Nasal swab samples were collected from both HIV positive and HIV negative and tested microbiologically by standard procedure. AST was based on Kirby disc diffusion method. MIC was determined by agar dilution method and results were interpreted according to CLSI (Clinical Laboratory Standards Institute) guidelines.

Results: Out of total 400 samples, 129 samples gave the positive growth result for *S. aureus*. Among those 129 *S. aureus* isolated, 52 were MRSA. From HIV positive individuals, out of 72 *S. aureus* isolated, 30 were MRSA and the rest were MSSA. Similarly in HIV negative, out of 57 *S. aureus* isolates, 22 were MRSA and rest were MSSA. HIV positive individuals showed 7.5% MRSA carrier rate and 5.5% MRSA carrier rate in HIV negative individuals, which in total contributed for 13% MRSA carrier rate. Almost all of the strains which were MRSA were MDR also. The antibiogram of all the MRSA isolates showed the similar results; all isolates were resistant to penicillin (100%), followed by oxacillin and cephalexin which were 90-96% and 75% resistant respectively

Conclusion: In conclusion, Ciprofloxacin was most effective drug besides vancomycin followed by erythromycin. Vancomycin resistant case was not found. So vancomycin can be considered as drug of choice for treating MRSA infections.

Keywords: MRSA, MSSA, MDR, HIV positive and HIV negative individuals.

INTRODUCTION

Methicillin resistant *Staphylococcus aureus* (MRSA) are strains of the *Staphylococcus aureus* that are resistant to the action of methicillin and related beta-lactam antibiotics (e.g. penicillin, oxacillin, amoxacillin etc). MRSA is also known as oxacillin resistant

Staphylococcus aureus (ORSA) and multiple resistant Staphylococcus aureus (Boyce, 2011). In the fall of 2007, the CDC (Center for Disease Control in Atlanta) reported that deaths due to MRSA were greater than those caused by AIDS. MRSA is rapidly becoming one of the most prevalent and menacing diseases of our time and increases the risk of time spent in a hospital.

Although the media discusses MRSA as a single new strain, in reality MRSA represents more than 1100 distinct strains of *S. aureus* (Wim and Neeling, 2005). *Staphylococcus aureus* is a ubiquitous organism in the human population residing in 30-40% of adults as the asymptomatic carriers. The rate of colonization and infection of *S. aureus* was found to be higher in HIV positive individuals than in HIV negative individuals. In HIV- positive individuals, inappropriate and irritational use of antibiotics may provide favorable conditions for resistant microorganisms to emerge, spread, and persist. Resistant is an emerging concern for the treatment of HIV infections, following the rapid expansion

in access to antiretroviral medicines in recent years (Harris *et al*., 2002).

METHODS

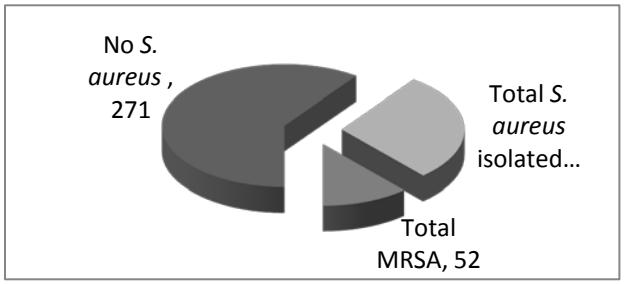
The study was carried out at Sukraraj Tropical and Infectious Disease Hospital from August 2012 to January 2013.

Nasal swab samples were collected from Individuals visiting the hospital. All the samples were first inoculated on MSA plates and incubated at 37^0C for 24 to 48 hours. Mannitol fermenting colonies of S. aureus surrounded by yellow zone due to production of acid (Davies and Zidac, 1997), were selected for further processing. The presumably identified isolates, Mannitol fermenting colonies from MSA were subcultured on NA and BA at 37^0C for 24 hours. For further identification, various confirmatory biochemical tests were performed by taking the isolates grown on NA (Baron et al., 1994). The different test done were Gram's staining, catalase test, oxidase test, cougulase test including both slide test and tube test, voges proskauer (VP) test, oxidation fermentation (OF) test. All the processing was done as per the standard methods. Antimicrobial susceptibility was determined by the Kirby-Bauer disc-diffusion method. The antibiotic discs used were Penicillin (30µg), cephalexin (30µg), Ciprofloxacin (5µg), Erythromycin (5µg), Oxacillin (1.25/23.75µg), Vancomycin (30 µg). Resistance was determined by agar disk diffusion method and results were interpreted according to CLSI (Clinical Laboratory Standards Institute) guidelines. Data were entered in Microsoft Excel and analyzed by SPSS version 16.0

RESULTS

During this study, total 400 nasal swab samples were collected. Among the total sample, 200 samples were from HIV positive individuals and remaining 200 samples were from HIV negative individuals. Out of total 400 samples, 129 samples gave the positive growth result for *S. aureus*. Among those 129 *S. aureus* isolated, 52 were MRSA. From HIV positive individuals, out of 72 *S. aureus* isolated, 30 were MRSA and the rest were MSSA. Similarly in HIV negative, out of 57 *S. aureus* isolates, 22 were MRSA and rest were MSSA. HIV positive individuals showed 7.5% MRSA carrier rate and 5.5% MRSA carrier rate in HIV negative individuals, which in total contributed for 13% MRSA carrier rate.

Fig: Distribution of total sample



Almost all of the strains which were MRSA were MDR also. The antibiogram of all the MRSA isolates showed the similar results; all isolates were resistant to penicillin (100%), followed by oxacillin and cephalexin which were 90-96% and 75% resistant respectively. However, the association of organism isolated with respect to different age groups and gender was found to be statistically insignificant (P>0.05). Even HIV positive individuals have no significant relationship with the duration of drug intake.

Table 1: Age wise distribution of *S. aureus* in both the study population

Age group	*S. aureus* in HIV positive		*S .aureus* in HIV negative	
(years)	Total sample	Positive isolates	Total sample	Positive isolates
0-10	6	2(33.33%)	8	2 (25%)
11-20	5	3 (60%)	32	9 (28.12%)
21-30	85	32(37.64%)	45	12(26.66%)
31-40	58	19(32.75%)	59	18(30.50%)
41-50	38	14(36.84%)	20	6 (30%)
51-60	6	1 (16.66%)	20	6 (30%)
61-70	1	0 (0%)	9	2(22.22%)
71-80	1	1 (100%)	7	2(28.57%)
Total	200	72 (36%)	200	57 (29%)

Table 2: Gender wise distribution on *S. aureus* in total samples

Sex	Types of individuals			
	HIV positive		HIV negative	
	Total isolates	Positive isolates	Total isolates	Positive isolates
Male	120 (30%)	40 (10%)	110 (27.5%)	32 (8%)
Female	80 (20%)	32 (8%)	90 (22.5%)	25 (6.25%)
Total	200 (50%)	72 (18%)	200 (50%)	57 (14.25)

Table 3: Relation of MRSA with duration of drug intake.

DODI (years)	MRSA		Total	P-value
	Sensitive	Resistant		
0-1	7	12(40%)	83	0.826
1-2	18	5(16.66%)	23	
2-3	23	5(16.66%)	28	
3-4	16	1(3.33%)	17	
4-5	17	3(10%)	20	
5-6	9	1(3.33%)	20	
6-7	3	0	3	
7-8	4	1(3.33%)	5	
More than 8	10	1(3.33%)	11	
Total	170	30	200	

Table 4: AST pattern of MRSA isolates in HIV positive individuals

Antibiotics	Sensitive	Resistance
Vancomycin	30	0
Oxacillin	1(3.33%)	29 (96.66%)
Cephalexin	7(23.33)	23 (76.66%)
Erythromycin	9(30%)	21 (70%)
Ciprofloxacin	9(30%)	21 (70%)
Penicillin	0(0%)	30 (100%)

Table 5: AST pattern of MRSA isolates in HIV negative individuals

Antibiotics	Sensitive	Resistance
Vancomycin	22(100%)	0(0%)
Oxacillin	2(9.09%)	20(90.90%)
Cephalexin	5(22.72%)	17(77.27%)
Erythromycin	7(31.81%)	15(68.18%)
Ciprofloxacin	10(45.45%)	12(54.54%)
Penicillin	0(0%)	22(100%)

DISCUSSION

In this study, the total nasal carrier rate from both HIV positive and HIV negative individuals was found to be 13 %, this finding was found in agreement with the internationally reported range of MRSA carriage 5.8 - 17.8% (Mulqueen *et al*, 2007). In spite of this agreement with international data, the rate was found higher among reported in Birgunj, Nepal, by Shakya *et al.*, 2010. The MRSA rate revealed by this study is almost equal to the MRSA rate reported by Niraula (2011) (12.9%) from Kathmandu, Nepal. But this rate was lower in comparison to reported by Anwar et al. (2004), Pakistan, in which it was reported to be 16.9% among healthy population. In similar kind of study, Rijal et al. (2008), in Pokhara, Nepal, reported 17.3% of nasal carriage rate of MRSA.

From the total 400 samples collected, only 129 samples were found to be positive for *S. aureus* growth. The positive Samples when screened for MRSA showed 52 were MRSA and rest was MSSA. Similarly, among those 52 isolated strains of MRSA, male accounted for 31 samples which contributed for 59.61 % MRSA nasal carrier rate and females for 21 samples that contributed for 40.38% MRSA nasal carrier rate. The highest prevalence rate of MRSA carrier in male was observed in comparison to the female in this study, which may be due to the higher number of nasal swabs collected from male individuals. The higher number of MRSA carrier in male is not statistically significant as the P- value is 0.74 (for significant relation P- value = 0.05), which clearly shows the relation is insignificant. Only one other study has found a marginally higher prevalence of MRSA carriage in males, although it was not statistically significant (Anwar *et al.,* 2004). Whether this is due to better commitment with infection control and hygienic practice of females, or other factors should be looked at in future studies.

In age wise carrier rate study, the total 400 samples were divided into two main categories as HIV positive one and another one as HIV negative one. In the first category, maximum *S. aureus* carrier rate was found in the age group 21-30 years and was followed by age group 31-40. Low carrier rate was found in age group 71-80 years. Likewise in HIV negative individuals, maximum *S. aureus* carrier rate was found in the age group 31-40 years, followed by age group 21-30 years and the lowest was found in similar to HIV positive 71-80 years age group. In the age group 21-30 years, maximum carrier was seen in case of HIV positive individuals. This might be due to frequent antibiotics intake, frequent hospital visit, tattooing or sexual behaviors.

Another study was done especially for HIV positive individual to know whether the duration on drugs intake (antiretroviral drug) makes any impact or not, revealed that there is no significant relation between ARV Therapy and MRSA carriage. The P- value calculated in this case was 0.82, which was highly insignificant. The maximum MRSA carrier rate (16.66%) was found in the group 1-2 years, similar in 2-3 years, followed by 0-1 years (40%) and others group were almost same (3.33%).

From this study, Vancomycin was found to be 100% sensitive. The finding is in accordance with those of the findings by Lamichhane *et al* (1999), Rajbhandari *et al* (2002), Thapa *et al* (2004) and Sapkota *et al* (2006). Similarly, the result also synchronizes with the findings of Khan *et al* (2007) and Kumari *et al* (2008). The most effective antibiotics after vancomycin was found to be ciprofloxacin 53(41.08%), followed by Erythromycin 44(34.10%). Penicillin was found to be of no use because almost 92% samples were resistant to it and even oxacillin was also very resistant with almost 83%. So it would be better if both of the drugs will not be prescribed for treating MRSA infections.

Similarly, the antibiogram study in HIV positive individuals also supported the above same result. This study also revealed that vancomycin was 100 % effective which was followed by others. Almost all the MRSA isolates were multi drug resistant. Even in HIV negative individuals, the result was same and all the 22 isolates of MRSA were multiple drug resistant. Previous recent study carried out in 2012 by Sapana Gaire also revealed that in HIV positive individuals, the most of isolates were resistant to Penicillin (100%) and so was in HIV negative individuals.

Conclusion

The rate of *S. aureus* colonization was higher in HIV positive individuals than the HIV negative individuals and so was the MRSA rate. Among different antibiotics used, penicillin showed almost 100% resistance and vancomycin was 100% sensitive. However other antibiotics such as ciprofloxacin and erythromycin also showed good sensitivity. The higher level of reduced susceptibility and report of full antibiotics resistant strains have increased a chance of clinical failure.

The study suggests that the nasal screening is a convenient and the useful techniques for the determination of the MRSA carriage. Since the infection is more in HIV positive it would be better if such system would be adapted by all the ART centers and relate hospitals.

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