International Journal of Research in Medical Sciences
Diwakar MK et al. Int J Res Med Sci. 2019 Feb;7(2):428-433
www.msjonline.org
pISSN 2320-6071 | eISSN 2320-6012
DOI: http://dx.doi.org/10.18203/2320-6012.ijrms20190347

Original Research Article

Resistance pattern of methicillin resistant *Staphylococcus aureus* among nasal isolates of HIV infected patients in a tertiary care hospital

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Received: 01 December 2018
Accepted: 29 December 2018

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ABSTRACT

Background: Patients infected with HIV have an increased risk of nasal *Staphylococcus aureus* carriage as well as consecutive staphylococcal infections and is a major reservoir for MRSA which is potential risk factors for community acquired MRSA. Knowing the Nasal carriage status of *Staphylococcus aureus* and their Antibiogram will be beneficial for effective management of these patients.
Methods: Nasal swab sample were collected from all the participants and processed for culture and identification of *Staphylococcus aureus* and their antimicrobial sensitivity. All the *Staphylococcus aureus* isolates were tested for Methicillin resistance by Oxacillin screen agar test, cefoxitin disc diffusion test and further confirmed by mecA gene PCR.
Results: In this study out of 220 HIV seropositive patients, 43.64% isolates were confirmed to be *S. aureus*, 18.75% MRSA and 81.25% were MSSA. Cefoxitin disc diffusion showed 100% specificity (95% CI; 97.05%-100.00%), 100% sensitivity (95% CI; 83.89-100.00%) and 100% accuracy (95% CI; 97.47% to 100.00%) while comparing with gold standard mecA gene PCR. Among the nasal carriers; males (60%) ware dominant on females (40%). 31-50 years age group was strongly associated with MRSA nasal carriage. None of the isolates were resistant against lenozolid, teicoplanin and vancomycin while ampicillin (75%), ciprofloxacin (62.5%), clindamycin (59.38%) and cotrimoxazole (53.13%) showed increased resistance against *S. aureus* nasal carriage.
Conclusions: Resistance among HIV positive persons for all antibiotics showed statistically significant while compared to control group. Cefoxitin disc diffusion can be used as surrogate agent for mecA gene detection.

Keywords: HIV seropositive, MRSA, Nasal carriage, Resistance

INTRODUCTION

Staphylococcus aureus is both a human commensal and a frequent cause of community as well as hospital acquired infections with substantial morbidity and mortality worldwide.[1] MRSA infections are therapeutic challenge for physicians because of the limited choice of antibiotics available and due to the possibility of concomitant drug resistance of the MRSA to other antimicrobials and. MRSA are also a challenge to patients in developing settings due to increased cost of care.[2]

Nasal carriage of *S. aureus* plays a key role in the development of *S. aureus* infections and is a major reservoir for MRSA, hence eradication of this microorganism from the nose can be an effective preventive measure, mostly in high risk group of patients.[1] Various studies has proven that the patients

infected with the human immunodeficiency virus (HIV) have an increased risk of nasal *Staphylococcus aureus* carriage and consecutive staphylococcal infections.[1,3] *Staphylococcus aureus* infections account for significant morbidity in human immunodeficiency virus (HIV)-infected patients.[4]

Reports by various authors from different geographical areas show a great diversity in the prevalence among the HIV seropositive population; ranges from 30%-76% prevalence of nasal carriage of *Staphylococcus* and 0-31% prevalence of MRSA carriage in HIV positive patient in India and abroad.[1,3-6] A 6-18-fold increased risk has been noted for MRSA-attributable infections in HIV-infected individuals compared to that of general population by Zervou FN et al.[7]

The prevalence of nasal carriage *Staphylococcus aureus* is influenced by the circulating *Staphylococcus aureus* in the community or in health care setup and the population of high-risk groups. Therefore, knowing the Nasal carriage status of *Staphylococcus aureus* in HIV positive patients will be beneficial for effective management. Therefore, we planned this study to determine the prevalence of MRSA nasal carriage in HIV seropositive patients and associated risk factors.

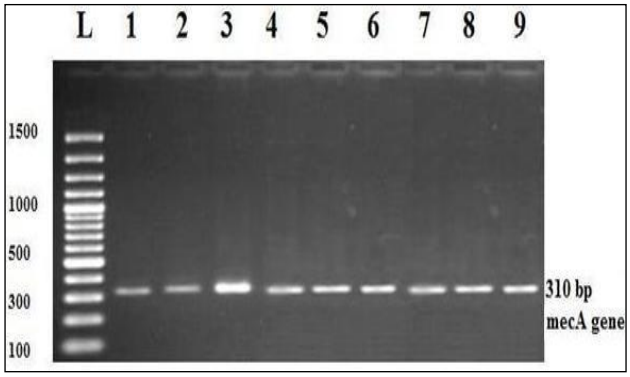
METHODS

Two hundred and twenty non-duplicate patients freshly diagnosed with HIV infection, attending the ICTC center at a tertiary care hospital were enrolled as case group after obtaining informed consent. Healthy people willing to participate in the study were included as control group for the study. Patients having diabetes, cancer, haemodialysis and other conditions leading to immunodeficiency were excluded from the study. Anterior nares swab sample from all the study population were collected according to standard microbiological protocol.

All the specimens were processed for culture and identification of methicillin resistance *Staphylococcus aureus* and their antimicrobial sensitivity. All nasal carriage specimens were cultured on blood agar and mannitol salt agar (Hi Media, New Delhi, India). *S. aureus* were identified and differentiated from related organisms as per conventional methods on the basis of colony morphology, Gram staining, catalase test, slide and tube coagulase, DNAse and mannitol fermentation following the standard procedures.[8]

All the *Staphylococcus aureus* isolates were tested for methicillin resistance by oxacillin screen agar test, cefoxitin disc diffusion test and further confirmed by mecA gene PCR. The antibiotic susceptibility patterns of all the *S. aureus* isolates were determined by Kirby Bauer disc diffusion method and interpreted according to the Clinical Laboratory Standards Institute guidelines.[9]

PCR was performed according to the protocol used earlier by Tiwari et al.[10] The bacterial DNA was extracted by spin column method as per manufacturer instruction. Five microlitres (5µl) of the extracted DNA was transferred to 20µl of PCR amplification mixture consisting of 2.5µl of PCR buffer, 2.5µl of MgCl2, 1.25U of *Taq* polymerase, 4µl of dntps and 1µl of each primer. The primer used in this study was reported earlier by Geha DJ et al prior to blast on NCBI database.[11] Forward primer was *mecA* 1 (5´ GTAGAAATGACTGAACGTCCGATAA 3´) and reverse primer was *mecA* 2- (5´ CCAATTCCACATTGTTTCGGTCTAA3´). PCR was performed as per condition used earlier by Tiwari et al and Geha DJ et al.[10,11] The PCR carried out with Initiation at 94°C for 4min, followed by 30 cycles of denaturation at 45sec at 94°C, annealing at 50°C for 45sec, and extension for 60sec at 72°C, with a final extension step at 72°C for 2min. 10µl of the PCR product of isolates was loaded in 2% agarose gel in TBE (0.089M Tris, 0.089M boric acid, 0.002 M EDTA) containing 0.5µl/ml of ethidium bromide and visualized by using UV transilluminator at 300nm. A DNA fragment of 310bp confirms the *mecA* gene (Figure 1).



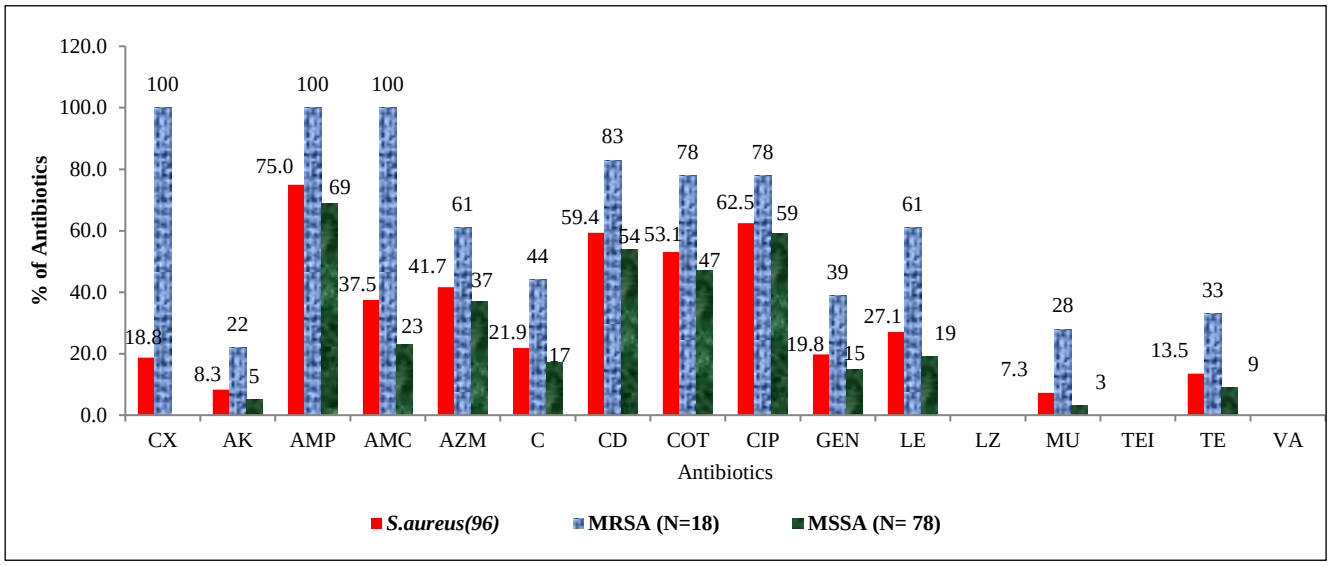
(L- Ladder of 100bp, samples- 1to9)

Figure 1: PCR product of 310 bp for mecA gene.

RESULTS

Nasal swab samples from 220 HIV seropositive patients and 220 healthy controls were collected with the help of sterile cotton swab. All the samples were screened for *S. aureus* colonization. 43.64% isolates were confirmed to be *S. aureus* Out of 220 HIV seropositive patients among whom 18.75% were identified as MRSA and 81.25% as MSSA. Similarly, among HIV negative patients; 21.82% isolates were confirmed to be *S. aureus* among which 6.25% isolates were identified as MRSA while 93.75% were MSSA. In this way the *S. aureus* colonization was observed as 32.7% overall.

Cefoxitin disc diffusion showed 100% specificity (95% CI; 97.05%-100.00%), 100% sensitivity (95% CI; 83.89% -100.00%) and 100% accuracy (95% CI; 97.47% to 100.00%) while comparing with gold standard mecA gene PCR.



(CX- Cefoxitin, AK- Amikacin, AMP- Ampicillin, AMC- Amoxicillin clavulanic acid, AZM-Azithromycin, C- Chlomphenicol, CD-Clindamycin, COT- Cotrimoxazole, CIP- Ciprofloxacin, GEN- Gentamycin, LE-Levofloxacin, LZ- Linezolid, MU-Mupirocin, TEI-Teicoplanin, TE- Tetracycline, VA- Vancomycin)

Figure 2: Antibiogram of nasal isolates of *S. aureus* among HIV positive patients.

Table 1: Comparison of antimicrobial resistance among nasal isolates of MRSA in HIV positive patients (cases) and normal healthy population (controls).

Antibiotic	Cases (%)	Control (%)	Odds ratio	95 % CI	P Value
CX	100	100	1	0.019-50.89	-
AK	22	0	57.6115	3.44-964.60	0.005
AMP	100	100	1	0.02-50.89	-
AMC	100	100	1	0.02-50.89	-
AZM	61	67	0.7704	0.43-1.37	0.37
C	44	33	1.5952	0.89-2.83	0.11
CD	83	67	2.4047	1.23-4.68	0.01
COT	78	67	1.7463	0.93-3.28	0.08
CIP	78	67	1.7463	0.93-3.28	0.08
GEN	39	33	1.2981	0.72-2.31	0.38
LE	61	33	3.1756	1.78-5.66	0.0001
LZ	0	0	1	0.02-50.89	-
MU	28	0	79.0138	4.75-1315.47	0.002
TEI	0	0	1	0.02-50.89	-
TE	33	0	57.6115	3.44-964.60	0.005
VA	0	0	1	0.02-50.89	-

Among the *S. aureus* nasal carriers; males (60%) was dominant on females (40%). Similarly, male (72%) had more MSSA carriage than females (28%). The nasal MRSA was observed highest in mature age group (31-40 years, 44%; 41-50 yrs, 33%) while least was observed in old age patients (51-60 years; 6%). Nasal isolates were found highest in lower middle class (SA-54%, MRSA-61%) while lowest in upper class people (SA-2%, 0 MRSA).

The maximum HIV positive patients were found who were directly related with private jobs and housewives. The farmers were also showed a high number of positivity to HIV. The minimum HIV positive patients were recorded who had directly government employed. Highest MRSA was found in age group of 31-50 yrs while least in <30 yrs age group and in more than 50 yrs of age group. However, statistically no significant difference was observed between rural and urban habitat

among HIV positive patients; rural habitat had more (61%) MRSA isolates.

Among 96 *Staphylococcus aureus* isolates of case group, 18.75% were identified as MRSA while 81.25% as MSSA. None of the isolates were resistant against lenozolid, teicoplanin and vancomycin while ampicillin and ciprofloxacin showed maximum resistance. Resistance for all antibiotics showed statistically significant while compared to control group. Similarly, among control group; 6.25% *S. aureus* were identified as MRSA and 93.75% as MSSA. The rate of resistance was found highest for ampicillin (55.45%) and least for linezolid, teicoplanin and vancomycin. For these three antibiotics zero resistance was seen among nasal isolates. The antimicrobial susceptibility pattern of nasal isolates among HIV positive patients is illustrated in Figure 2.

All the commonly used 16 antibiotics was found statistically significant resistant for MRSA as compared to MSSA in HIV positive patients. The comparison of antimicrobial resistance among nasal isolates of MRSA in HIV positive patients and normal healthy population is demonstrated in Table 1.

The logistic regression analysis for antimicrobial resistance among nasal isolates of MRSA in HIV positive patients (cases) and normal healthy population (controls) showed significant difference for amikacin, clindamycin, levofloxacin, mupirocin and tetracycline while no significant difference was observed for azithromycin, chloramphenicol, cotrimoxazole, ciprofloxacin, gentamycin.

DISCUSSION

In 1982, first report of CA-MRSA was seen in UK among drug abusers. Since that time increased level of MRSA is being reported all over the world including India.[7,12,13] It is known that Patients colonized with MRSA may have more chance to get MRSA.[14]

Various studies have been proved that HIV is a risk factor for colonization of *S. aureus* and MRSA.[3,15,16] In this study, among the newly diagnosed HIV positive population, *S. aureus* nasal carriage was found around 43.64 % in which 18.75% were MRSA whereas the carriage rate was 21.82 % and 6.25% for *S. aureus* and MRSA in control group. This finding approves HIV as a risk factor for nasal colonization.

Our prevalence of nasal carriage *S. aureus* was near about similar as reported by various others authors.[17,18] The Contrast finding was also reported by various authors.[3,19,20]

Higher prevalence may be due to the immunocompromised status, frequent contact with both health care and community settings and frequent exposure to antibiotics; becoming them colonized with resistant strains which is also proposed by Hidron AI et al.[4]

Cefoxitin disc diffusion showed 100% specific (95% CI; 97.05%-100.00%), 100% sensitive (95% CI; 83.89%-00.00%) and 100% accurate (95% CI; 97.47% to 100.00%) method while comparing with gold standard mecA gene PCR. Our finding was also in consideration with various other author's findings.[21,22]

Rural population was at more risk for nasal carriage among HIV seropositive patients (statistically non significant) in the present study. Females (20.97%) account for more (statistically non significant) nasal carriage MRSA as compared to males (14.71%). The similar finding was also observed by some authors.[20,23] However, the contrast finding was also reported.[24,25] The higher MRSA colonization rate in females in this geographical region may be due to lack of awareness to their health and hygiene. It's a fact that about 65% of the patients (75% females) have received only a primary level education which might be a factor constructive for keeping good hygiene and resulting in more MRSA colonization.

The nasal carriage was found highest in mature age group; 40% *S. aureus* and 44% MRSA among 31-40 years age group and least was considered among old age group (51-60 years) patients in which 7% *S. aureus* and 6% MRSA was found. However, *S. aureus* colonization among cases and controls shows no statistically significant correlation between age and carriage rate (P= 0.663; 95% C.I for EXP (B) 1.005 = 0.983- 1.028) in binary logistic regression analysis. But, in contrast to MRSA carriage; age group of 31-50 years was strongly associated with MRSA nasal carriage. Findings of other authors also justify the age group having highest carriers.[6,24] However, non-significant correlation between age and carriage has been also reported.[26]

The majority of MRSA carriers belong to upper lower and lower middle class (77.08% *S. aureus*; 94.44% MRSA) in this study. Whereas none of the upper class patients having MRSA nasal carriage. This may be due to habitat status, poor hygienic, poor nutrition and environmental condition of lower/upper lower class population. This result has been also validated by a study performed by Kumari N et al.[27] However, the finding diverge from Chatterjee SS et al, which reported high class population as more affected (16.67%) than lower (7.23%) and middle (3.74%) class population. This variation may be resultant of sample size variation among different socio-economical classes.[20]

In this present study, Chi square test indicated statistically significant resistance for all antibiotics against MRSA (P=<0.05) in HIV infected study group as compared to MSSA isolates of the anterior nares. While comparing the antibiotic resistance pattern of MRSA between cases and control in the present study none of

the isolates was resistant for linezolid, teicoplanin and vancomycin in both cases and controls. Resistance against other non-beta lactam antibiotics like azithromycin, chloramphenicol, cotrimoxazole, ciprofloxacin and gentamycin didn't show any statistically significance difference for MRSA isolates among cases and controls. However, statistically significance difference was observed against amikacin, levofloxacin, mupirocin, tetracycline and clindamycin. In our study there was no resistance observed for vancomycin, linezolid and teicoplanin among study population. Various researchers also found 100% sensitivity for the same.[3,4,28] However, few studies has reported vancomycin resistance (VRSA) in MRSA strains, while some researchers found increased MIC against vancomycin among nasal isolates.[10,29]

Other antibiotics like amikacin (78%), mupirocin (72%), doxycyclin (67%), gentamycin (61%) and chloramphenicol (56%) showed good sensitivity whereas clindamycin (17%), co-trimoxazole (22%) ciprofloxacin (22%), levofloxacin (39%) and erythromycin (39%) showed less sensitivity against MRSA strains among HIV positive persons.

Hidron AI et al, found resistance for clindamycin (32%); doxycycline (10%); gentamycin (23%); clindamycin (92.5%); oxa (100%); quineprestin (83%); rifampicin (20%); cotrimoxazole (21%); vancomycin (0%) among nasal isolates of MRSA.[4] High resistance for cotrimoxazole (76.9%), ciprofloxacin (76.9%) and erythromycin (69.2%) was also observed.[6] Similarly, various authors reported different resistance pattern for their study area and population.[26,27,30,31] The variation in antibiotic resistance pattern against MRSA nasal carriage was observed in the findings of different researchers according to the local antibiotic policies and various factors.

Increasing resistance among nasal MRSA may create difficulty to manage infections and may cause more complications in HIV patients. The reason for the higher colonization rate and increased resistance among MRSA isolates in HIV patients are unclear but could include the diminished immune response and factors such as frequent contact with both hospital and community settings and frequent exposure to antibiotics, leading to a greater likelihood of becoming colonized with resistant strains. Based on the findings of this study, we recommend the use of chloramphenicol, tetracycline, mupirocin, gentamycin, amikacin, vancomycin, teicoplanin and linezolid for the management of MRSA infections among HIV infected patients. Additionally, screening for carriage frequency and decolonization must be considered by hospital administration.

CONCLUSION

A higher colonization rate and increased resistance among MRSA isolates is seen in HIV seropositive patients as compared to HIV negative persons. The reasons for the elevated rates may be multifactorial, but probably related to lifestyle behaviors (e.g. high-risk sexual activities and drug abuse, underlying immune dysfunction, higher rates of antibiotic use and frequent exposure to healthcare settings). The accurate relationship between HIV infection and MRSA colonization has yet to be fully elucidated and further research is required. Screening of MRSA carriage status may be considered to reduce infections caused by MRSA in HIV-infected individuals.

Funding: No funding sources
Conflict of interest: None declared
Ethical approval: The study was approved by the Institutional Ethics Committee

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Cite this article as: Diwakar MK, Goyal A, Goyal S. Resistance pattern of methicillin resistant *Staphylococcus aureus* among nasal isolates of HIV infected patients in a tertiary care hospital. Int J Res Med Sci 2019;7:428-33.