

Nasal Colonization of Methicillin and Inducible Clindamycin Resistant Staphylococci and Cd4 Correlation in HIV Seropositives

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Abstract- Background: HIV- infected patients are at high risk of colonization and infection with methicillin resistant *Staphylococci* over the past decade. Increasing non- β -lactam antimicrobial resistance among methicillin resistant *Staphylococcal* clones, particularly to clindamycin, may complicate the efforts to manage infections in the community.

Methodology: Nasal swabs from 200 HIV patients were cultured. *Staphylococcal* isolates were tested for methicillin resistance by Cefoxitin disk diffusion test & inducible clindamycin resistance by 'D test' as per CLSI guidelines. CD4 counts of the patients were determined and analyzed.

Results: Among the screened HIV patients prevalence rate of *S. aureus* was 45.5% (91/200) out of which 27% (54/200) was MRSA, CoNS was 25.5% (51/200) with 13% (26/200) being MRCoNS. Inducible clindamycin resistance was detected in 16.66% MRSA, 18.91% MSSA, 19.23% MRCoNS and 16% MSCoNS. The CD4 counts ranged from 22 to 1235 cells/mm³. Statistical significance was not observed between CD4 values and nasal *Staphylococcal* colonization.

Conclusion: In HIV patients, we should have a high level of suspicion regarding methicillin resistant *Staphylococci*, irrespective of patients' CD4+ T lymphocyte counts.

Index Terms- Methicillin Resistant *Staphylococci*; Inducible Clindamycin Resistant *Staphylococci*; nasal colonization; HIV; CD4 count

I. INTRODUCTION

Staphylococcus aureus (*S. aureus*) is the most commonly isolated human bacterial pathogen [1] and is the leading cause of gram positive bacterial infections [2]. *Staphylococcal* infections have clinical range from minor skin infections to severe life-threatening infections [3]. The primary reservoir of *S. aureus* is the anterior nares [4]. The relationship between colonization with *S. aureus* and human immunodeficiency virus (HIV) infection is of particular interest due to the associated morbidity and mortality [5].

Antimicrobial drug resistance in *S. aureus* arose early after the development of antimicrobial agents and continues to evolve and this limits the choice of potentially efficacious agents [6].

The emerging Methicillin resistant *Staphylococcus aureus* (MRSA) is a problematic pathogen in the world [7]. Life-threatening sepsis, endocarditis, and osteomyelitis caused by MRSA have been reported [8]. Carriage of MRSA precedes endogenous MRSA infections [9]. Since resistance to multiple

antibiotics among MRSA isolates is very common, there is a possibility of extensive outbreaks, which may be difficult to control [8].

This has led to renewed interest in the usage of clindamycin which belongs to Macrolide-Lincosamide-Streptogramin B (MLSB) antibiotics to treat *S. aureus* infections. However, widespread use of these antibiotics has led to an increase in the number of *Staphylococcal* strains acquiring resistance to this group of antibiotics [10]. Concern over the possibility of emergence of clindamycin-resistance during therapy which may cause treatment failure has discouraged some clinicians from prescribing this agent. However simple laboratory testing (e.g. the erythromycin- clindamycin 'D-zone' test) can separate strains that have the genetic potential (i.e. the presence of *erm* genes) to become resistant during therapy, from strains that are susceptible to clindamycin [11].

MRSA infections in HIV patients are reported to be 6 to 18 fold higher than in the general population [12]. There is a particular concern about life-threatening invasive MRSA infections, increased risk for persistent nasal colonization and recurrent infections at low CD4+ cell counts [13, 14].

As the role of nasal colonization with methicillin resistant & inducible clindamycin resistant *Staphylococci* is not well established, we decided to undertake a prospective study in HIV seropositive individuals.

Our objectives were to determine the prevalence of nasal *Staphylococcal* colonization, and determine methicillin resistance & inducible clindamycin resistance of nasal *Staphylococcal* isolates and to co-relate with the CD4 count of these patients.

II. MATERIALS AND METHODS

The population for this study was drawn from the HIV seropositive patients who visited the ART centre, Krishna Rajendra Hospital, attached to Mysore Medical College and Research Institute (MMC & RI), Mysore, South India for a routine clinic visit and who were willing to participate in the study. Study was conducted in the Department of Microbiology, MMC&RI and was approved by the institutional ethical committee.

200 patients were enrolled in the study. Informed consent was taken from the patients. Patient details with relevant history was recorded in the pro forma.

Nasal samples were collected from both the anterior nares using separate sterile culture swabs. Swabs were inoculated on 5% sheep blood agar and incubated at 37°C for 24 hrs. Bacterial

growth was identified as *Staphylococci* by standard procedures [15].

Methicillin resistance was detected by Cefoxitin disk diffusion test. A suspension of each isolate was prepared with the turbidity equal to 0.5 McFarland standard and lawn cultured onto Mueller Hinton agar plate. A 30 µg cefoxitin disc was placed and incubated at 37°C for 24 hrs. The zone of inhibition was measured. Results were interpreted according to the criteria of Clinical and Laboratory Standards Institute (CLSI) [16]. The zone of inhibition of *S. aureus* \square 21 mm and Coagulase negative *Staphylococci* (CoNS) \square 24 mm were considered as methicillin resistant.

Inducible clindamycin resistance (ICR) was tested by 'D test' as per CLSI guidelines [16]. Mueller Hinton agar plate was inoculated with 0.5 McFarland standard bacterial suspension. Erythromycin (15µg) and clindamycin (2µg) discs were placed at a distance of 15 mm (edge to edge). Following overnight incubation at 37°C, ICR was detected by flattening of zone (D-shaped) around clindamycin in the area between the two discs.

All the *Staphylococcal* isolates were tested for Linezolid (30µg) susceptibility by Kirby Bauer disk diffusion method.

CD4 counts of these patients were determined by flow cytometry (FACS caliber) and analyzed.

III. STATISTICAL METHODS

Summary statistics was done by measuring proportions with 95% confidence Interval (CI), mean and Standard Deviation. Inferential statistics was done using chi-square test/fisher exact test for identifying difference in proportions. Odds Ratio with 95% CI is calculated. Independent *t* test is used for identifying difference in means between colonized and non-colonized patients. All the analysis was done by using SPSS version 13.

IV. RESULTS

Two hundred patients were enrolled in the study: median age 34.19 ± 9.216 years (range 3 – 60). There were 74 males and 126 females.

Staphylococci was isolated from 142 (71% CI 70.9-71.0) patients. MRSA and MRCoNS were detected in 54 (59.34% CI 59.3-59.4) and 26 (50.98% CI 50.9-51.0) patients respectively [Table 1].

The prevalence rate of *S. aureus* among HIV patients screened was 45.5% (91 out of 200) with 27% (54 out of 200) being MRSA and CoNS 25.5% (51 out of 200) with 13% (26 out of 200) MRCoNS.

ICR was detected in 16.66% of MRSA, 18.91% of MSSA, 19.23% of MRCoNS & 16% of MSCoNS isolates [Table 2].

All the *Staphylococcal* isolates were sensitive to Linezolid.

MRSA colonization was found more in patients >45 years of age, MRCoNS in 31-45 years, ICR in <30 years [Table 3].

The CD4 counts of patients ranged from 22 to 1235 cells/mm³. The odds ratio for MRSA, MSSA, MRCoNS, MSCoNS, ICR colonization were 0.7, 0.7, 0.4, 0.9, 0.9 respectively when CD4 >200 compared to lower CD4 counts [Table 4]. However

none of these was significant, indicating no role of CD4 count in the colonization of MRSA, MSSA, MRCoNS, MSCoNS and ICR *Staphylococci* in the study sample.

V. DISCUSSION

Nasal colonization with *S. aureus* is a significant risk factor for serious infections [17]. Patients colonized with MRSA when compared with those with MSSA are more prone to subsequent infections [18]. So colonized *S. aureus* elimination may reduce the rate of subsequent invasive infections [17].

The prevalence of *S. aureus* nasal colonization in HIV-infected patients is between 0% and 17% [4]. The present study prevalence rate is 45.5% which is higher compared to 33.6% & 23% in the previous studies [19, 20]. MRSA colonization was observed to be 27% in our study. Other study reports of colonization are 3%, 10.3%, 8.4% [20, 4, 21]. The reason for the higher colonization rates observed are unclear, but could include factors such as frequent contact with health care settings and frequent exposure to antibiotics, leading to colonization with resistant strains [12].

In immunocompromised hosts, CoNS have become increasingly recognized as agents of clinically-significant nosocomial blood stream infections [22]. Previous study showed that 58% of bacterial blood stream infections in HIV-infected adults were due to CoNS [23]. In the current study the prevalence rate of CoNS was 25.5% and MRCoNS was 13%. Nasal carriage of MRCoNS is also highly prevalent, but its dynamics has been less investigated [24].

The *Staphylococcal* colonization was not statistically significant in different age groups. MRSA colonization was found to be statistically significant in male and female distribution (p value 0.02).

In the current study the association between *Staphylococcal* nasal colonization and CD4 count was evaluated. It was found that there was no statistically significant association between them. This is consistent with a previous study which reported that MRSA colonization was independent of CD4+T lymphocyte counts [21]. However in one study *S. aureus* colonization rate among HIV positive individuals increased as the CD4 count decreased [19]. Mean CD4 count of our study was 408 cells/µL. Previous study showed median CD4 cell count of 252 cells/µL and it was not associated with *S. aureus* colonization [25].

Staphylococcal colonization in patients on highly active antiretroviral therapy (HAART), antibiotics, hospitalization and skin infections was not found to be statistically significant [Table 4]. A previous study also reported that MRSA colonization was independent of antiretroviral treatment status, prior history of MRSA infection and chronic skin conditions. Previous antibiotic use was the only statistically significant risk factor for MRSA carriage [21]. Another study reported that MRSA colonization was associated with lower CD4 cell count, not receiving current or recent antibiotics, history of prior MRSA or MSSA infection [4].

In the present study MRSA colonization was observed in 62.5% of patients with tuberculosis and 25.52% of patients without tuberculosis and it was found to be statistically significant (p value 0.04).

Due to the emergence of resistance to antimicrobial agents accurate drug susceptibility data of the microbe is an essential factor in making appropriate therapeutic decisions [26]. Increasing non- β -lactam antimicrobial resistance among MRSA clones, particularly to clindamycin, may complicate efforts to manage infections in the community [1]. Clindamycin can be administered in patients with clindamycin-susceptible strains if ICR is excluded [17]

In this study ICR was detected in 16.66% of MRSA, 18.91% of MSSA, 19.23% of MRCoNS & 16% of MSCoNS isolates. Earlier study has reported lower susceptibility of MRSA isolates to commonly used antibiotics such as clindamycin, erythromycin, and ciprofloxacin. 42.9% of all MRSA isolates were resistant to clindamycin [21]. We could not get many ICR studies from nasal colonizers. Previous studies reported 19% incidence of clindamycin resistance in MRSA isolates from other *Staphylococcal* infections in children and young adults with HIV infection [27]. Another study reported 63% resistance in MRSA isolates from skin and soft tissue infections (SSTIs) in men who have sex with men [28]. Given the possible increasing rate of resistance of MRSA isolates, clindamycin should be used with caution if local antibiograms suggest high-grade resistance of MRSA isolates [12]. Compared to these studies our rate of ICR is low, probably because other studies are in isolates from infections.

Duration of MRSA carriage and risk of invasive infections in the study-group could not be determined as we did not follow up these patients. We could not confirm these strains by genotypic methods. The two important resistance strains we come across during treatment of *Staphylococcal* infection has been detected by simple methods which can be easily adopted by any laboratory as it is not technically demanding. It is economical, with good reproducibility. Detection of these strains in HIV sero-positives will help to take preventive measures to avoid invasive infections in this high risk group.

In several studies, the elimination of nasal carriage reduced the incidence of *S. aureus* infections [29]. Although optimal regimens for decolonization have not yet been established by clinical trials, most clinicians use topical agents including intranasal topical mupirocin administered b.i.d along with chlorhexidine body wash daily for 5- 7 days [30]. The results of a previous study suggested that a five-day course of mupirocin applied on a monthly basis can suppress *S. aureus* colonization over an extended period of time in high-risk individuals like HIV patients [31]. Although mupirocin resistance is described, the rate of resistance appears low. Other agents for decolonization include a variety of oral antibiotics such as rifampin combined with trimethoprim-sulfamethoxazole, minocycline or doxycycline [30].

VI. CONCLUSION

Universal surveillance regimens are being introduced in hospitals to control MRSA infections. Our findings suggest that targeted monitoring of HIV infected individuals for *Staphylococcal* carriage status of resistant strains may be an important clinical and public health approach. In HIV patients, we should have a high level of suspicion regarding MRSA, irrespective of patients' CD4+T lymphocyte counts. As these

patients are ambulatory there may be a risk of transmission of these resistant strains in the community. Future studies are needed to address the role of nasal colonization on subsequent infections and the utility of decolonization in this high risk group.

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