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ANALYSIS OF VANCOMYCIN RESISTANCE AMONG METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN HIV PATIENTS SUFFERING FROM RESPIRATORY ILLNESS

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ABSTRACT

Methicillin Resistant *Staphylococcus aureus* (MRSA) is responsible for a wide number of life threatening infections globally. The association of methicillin resistant *Staphylococcus aureus* in respiratory illness is considered as a serious threat for HIV patients. The vancomycin is widely used drug for the treatment of MRSA. The increased risk of vancomycin resistance among MRSA is a global challenge. In this study the level of vancomycin resistance with minimum inhibitory concentration (MIC) was observed among MRSA strains, isolated from HIV patients suffering from respiratory illness. Total 62 throat swab samples were collected and cultured on mannitol salt agar plates, 54 samples (87%) were cultured successfully. Among them, 43 samples (79.60%) were characterized as *Staphylococcus aureus* by biochemical tests. 5 samples (11.60%) were considered as MRSA showing MIC > 6mcg for cefoxitin antibiotic. MecA gene was detected in all MRSA positive strains by PCR. The MIC values of vancomycin and cefoxitin for MRSA strains were analyzed by E test (Epsilometer test) using Vancomycin-Cefoxitin E-test strips (Ezy MIC strips, Himedia). The MIC values for vancomycin were found between 0.75 to 3.0 mcg/ml, while cefoxitin MICs were observed between 8.0 to 24.0 mcg/ml for MRSA isolates. Only 1 MRSA sample (20%) was found vancomycin Intermediate while remaining 4 samples (80%) were found sensitive to vancomycin.

KEYWORDS: Methicillin resistant *Staphylococcus aureus*, Minimum inhibitory concentration, Respiratory illness, Mortality.

INTRODUCTION

Bacterial pulmonary infections are a major cause of mortality among HIV patients.[1] *S. aureus* is present on the skin and mucous membranes of the respiratory tract of healthy individuals,[2] which has been associated with several infections such as skin infections, bacteraemia, osteomyelitis, diarrhea, pneumonia, septicemia and urinary tract infections.[3,4,5,6] HIV and AIDS patients are more susceptible to these infections with high rates of morbidity and mortality.[7,8] *Staphylococcus aureus* is a known cause of infection among the Immunocompromised population.[9,7] The decline in CD4 cell count increases the risk of respiratory tract infections such as bacterial pneumonia in HIV patients.[1,10] First MRSA isolate was detected in 1961 and is still a well known clinically important pathogen.[11] Penicillin resistance may also lead to multiple drug resistance for other classes of antibiotics as aminoglycosides, macrolides, tetracycline, etc.[12] After the introduction of highly active anti retroviral therapy (HAART), a significant reduction in opportunistic infection was observed but bacterial pneumonia is still considered as a serious problem in AIDS.[13] The vancomycin, member of a glycopeptide group of antibiotics is considered as a best alternate for the treatment of MRSA associated infections. However, there are various previous studies indicating the emergence of vancomycin resistance among the MRSA isolates.[14] These vancomycin resistant *Staphylococcus aureus* (VRSA) and vancomycin intermediate *Staphylococcus aureus* (VISA) might cause serious threat for the coming world. The first report regarding reduced susceptibility to vancomycin in *S. aureus* was reported in 1996.[15] Currently there are many studies support a reduction in vancomycin susceptibility globally, including India.[16,17,18,19] The higher rate of emergence of VRSA has made the antibiotic therapy for *S. aureus* associated disease a global challenge.[20,12] The aim of this study is to analyze the level of vancomycin resistance among MRSA isolates of HIV patients suffering from respiratory illness.

MATERIALS AND METHOD

1. Sample collection: 62 throat swab samples of HIV patients were collected from Sanjay Gandhi Post Graduate Institute, Lucknow and Ram Manohar Lohia hospital during 2014-2015.

2. Bacterial isolation and Identification: Samples were cultured on Mannitol Salt agar plates and incubated at 37°C for 24 hours. Isolates were identified as *S. aureus* based on morphological analysis and biochemical reactions as per the standard protocol.[21]

3. Bacterial DNA Isolation: Bactetrial cell were pelleted by centrifugation of 200 µl broth culture at 4000 RPM for 15 min. Cells were resuspended in 45 µl of H_2O, 5 µl of lysostaphin (sigma) solution was added and incubated at 37°C for 10 min. 5 µl of proteinase K solution with 150 µl 0.1 M Tris HCL was added and incubate for 10 min. Samples were kept in a water bath for 5 min at 95°C followed by phenol/chloroform extraction.[22] DNA extracts were stored at -20 °C.

4. PCR amplification: Isolates were screened by PCR for the presence of the mecA gene using specific primer- forward 5'-AAAATCGATGGTAAAGGTTGGC-3' and reverse 5'-AGTTCTGCAGTACCGGATTTGC-3' resulted in amplification of the 533-b p product.[23] The thermal cycling conditions: an initial denaturation at 95°C for 5 min, followed by 40 cycles of denaturation at 95°C for 30 Sec, annealing at 50°C for 30 Sec and extension at 72°C for 90 Sec, with final extension at 72°C for 10 min. Followed by agarose gel electrophoresis. DNA bands were observed under UV gel imaging system (Bio-Rad).

5. E-test (Epsilometer test): Minimum inhibitory concentrations (MICs) of vancomycin and cefoxitin antibiotics were determined using Vancomycin - Cefoxitin E test strips (Ezy MIC^{TM}, Himedia). MIC values were determined at the interception point of the zone of inhibition around the E-test strips as per manufacturer's guidelines.

RESULTS AND DISCUSSION

In this study among total 62 samples, 35 (56.4%) were male and 27 (43.6%) were female. 54 (87%) samples were cultured successfully. Among them, 43 (79.6%) samples were considered as *Staphylococcus aureus* in biochemical tests. Only 5 (11.60%) samples were confirmed as methicillin resistant, showing MIC > 6 mcg/ml for cefoxitin antibiotic in E Test (figure -1), while molecular detection of mecA gene was done in all 5 MRSA samples by PCR (figure-2).

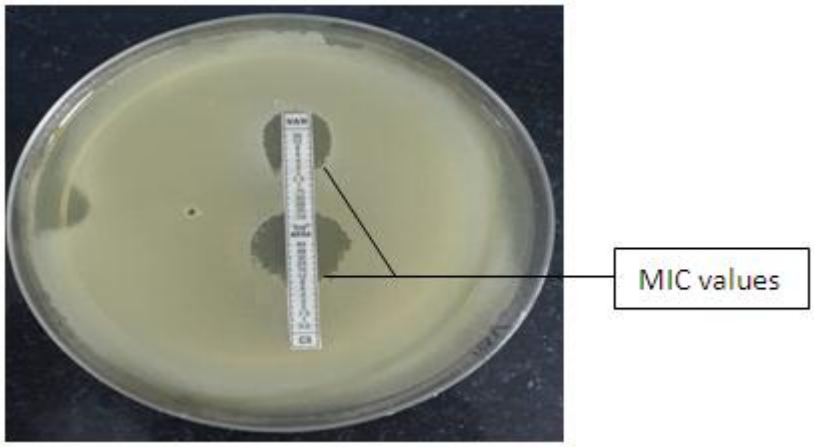

Figure 1: Vancomycin - Cefoxitin E-strip showing a zone of inhibition at different concentrations.

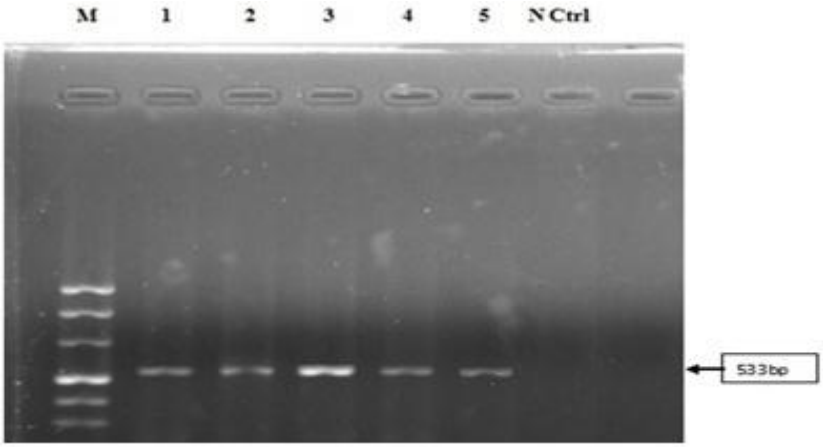

Figure 2: Agarose gel image showing polymerase chain reaction amplification of mecA gene.

According to a previous study methicillin resistant *Staphylococcus aureus* is very common pathogen associated with hospital associate respiratory tract illness in HIV patients. It is found associated in 25 % cases of pneumonia in HIV patients, while 65% of them are MRSA [4]. The MIC values of vancomycin and cefoxitin antibiotics against MRSA strains were found between 0.75 to 3 mcg/ml and 8 to 24 mcg/ml respectively using E test (Table-1, Figure-3a, 3b). Both agar dilution and E test methods are being used for analyzing MIC of antibiotics. Some studies reported that MICs observed by E test are higher than the MICs calculated by serial dilution assay [24]. In this study only 1 MRSA isolate (20%) was found vancomycin intermediate showing MIC value - 3 mcg/ml, remaining 4 isolates (80%) were found vancomycin sensitive (MIC value < 3mcg/ml). Few surveillance studies in India are supporting this data showing 100% sensitivity of MRSA isolates against vencomycin

antibiotic [18]. While data of a surveillance study of *S. aureus* isolates from the Europe and USA reported a very low prevalence (<0.3%) of vancomycin intermediates among total *S. aureus* isolates (>300,000) [16,25]. A recent study from South India indicated a high prevalence of VRSA and VISA among hospitalized patients [12].

Table-1 MIC values (mcg/ml) of vancomycin and cefoxitin antibiotics by E test

Isolates (MRSA)	MIC value (mcg/ml)	
	Vancomycin	Cefoxitin
MRSA-1	3.0	24
MRSA-2	0.75	8
MRSA-3	1.5	8
MRSA-4	2.0	16
MRSA-5	1.5	8

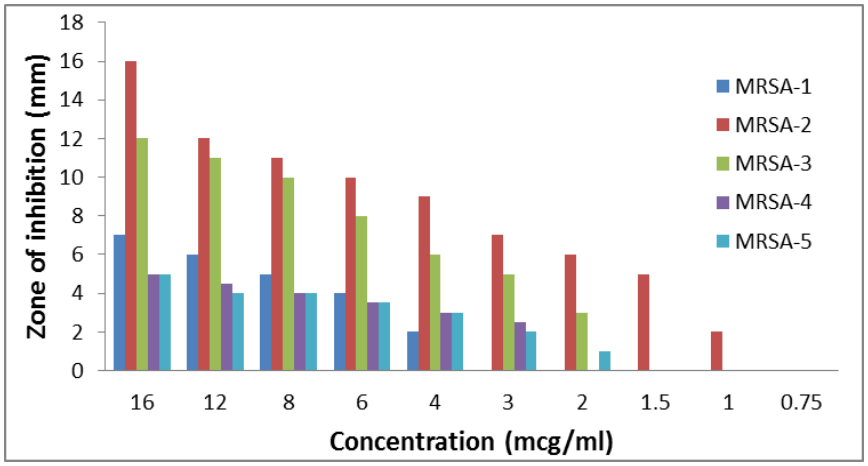

Figure 3a: Vancomycin concentration on E strip and respective zone of inhibition size (mm).

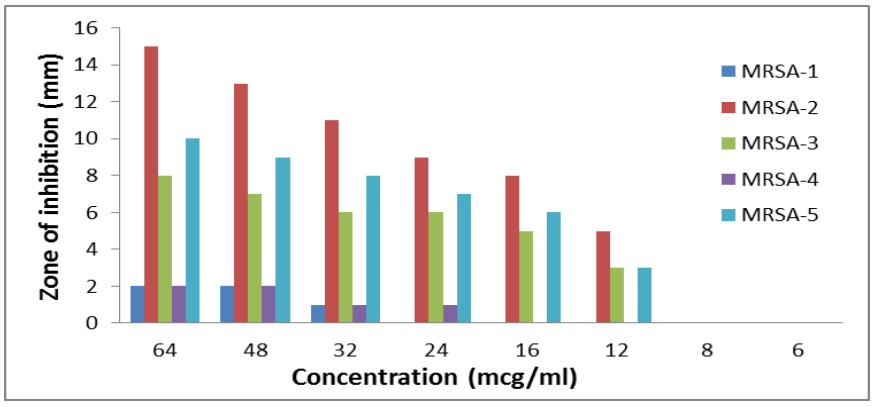

Figure 3b: Cefoxitin concentration on E strip and respective zone of inhibition size (mm).

CONCLUSION

The results conclude that *Staphylococcus aureus* is highly prevalent in HIV patients suffering from respiratory illness, The methicillin resistance in MRSA isolates might be associated with the presence of mecA gene. No MRSA is found resistante to vancomycin antibiotic. This study supports the use of vancomycin as treatment of choice against MRSA associated respiratory illness among HIV patients.

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