ORIGINAL ARTICLE

Suppurative Infections in Hospitalized Patients – An Ongoing MRSA Threat

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ABSTRACT

Background: Penicillin resistance among *Staphylococcus aureus* commonly encountered in the hospital admitted patients. Detection of antibiotic sensitivity in hospital acquired methicillin resistant *Staphylococcus aureus* infections is important as it has great influence on empiric antibiotic prescription, successful control of infection, prevention of spread of disease and successful patient management. This study aimed to detect the frequency of HA-MRSA from pus samples in a hospital setup with assessment of their antibiotic susceptibility patterns.

Method: A cross-sectional study was conducted in the Microbiology department of Basic Medical Science Institute, JPMC, Karachi from January 2015 until December 2015. Pus samples from surgical site wounds, skin lesions, abscesses from surgical and medical wards and ICUs were collected. According to the standards given by CLSI 2014, MRSA testing of the samples was done and susceptibility testing for antibiotics was performed. Inducible clindamycin resistance was detected by D-Test. E Test determined the MIC (minimum inhibitory concentration) for vancomycin. The data was analyzed by SPSS version 16.

Result: Out of the 149 MRSA identified from the pus samples, 106 (71.14%) samples were HA-MRSA. The number of male patients was more than the female patients (67.66%). Out of the 106 HA-MRSA, 91(85.8%) were sensitive to TMP/SMX, 98(92.5%) to rifampicin, 12(11.6%) to gentamicin, 85(80.2%) to tetracycline, 11(10.4%) to erythromycin, 37(34.9%) to clindamycin, 20(18.9%) to ciprofloxacin, 106 (100%) to both vancomycin and linezolid.

Conclusion: HA-MRSA showed sensitivity to TMP/SMX and vancomycin making them effective drugs to use in combination in superficial infections. The drug linezolid also showed 100% sensitivity.

Keywords: *Staphylococcus aureus*; HA-MRSA; Surgical Site Wound; Antibiotic Sensitivity; Clindamycin.

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INTRODUCTION

Staphylococcus aureus is a gram-positive coccus arranged in clusters. It is identified from other staphylococcal species on the grounds of the gold pigmentation of its colonies and positive results of coagulase, mannitol-fermentation, and deoxyribonuclease tests. Identification of this microorganism remains important, as it is the key factor acting in various severe infections like bacteremia, pneumonia, scalded skin syndrome, superficial skin infections and toxic shock syndrome. Initially, penicillin was a strongly effective antibiotic against infections due to the microorganism *Staphylococcus aureus*. Within only a few years of the discovery of the antibiotic penicillin, resistance to this drug among S. aureus was detected[1]. In 1940s, the bacteria started producing penicillinase thereby decreasing the effectiveness of β lactam antibiotics. Methicillin resistance in patients who have hospital acquired *Staphylococcus aureus* (HA-MRSA) is an infection commonly encountered in those hospital enrolled patients who are admitted for more than 48 hours[2].

The definition of MRSA as put by CLSI[3,4] is that strain of *Staphylococcus aureus* that is resistant to a large group of antibiotics we know as β-lactams, that includes the antibiotics group penicillin and cepha-

losporins[5]. Methicillin resistance is due to the presence of gene, which is known as mec A gene. It is responsible for encoding a protein of 78 kD, a penicillin binding protein (PBP)-2a or PBP2' which have a low affinity for this large group of β-lactam antibiotics[6,7]. According to various researches, this seems to be due to the result of injudicious use of common antibiotics used for a prolonged period of time as a result of which the bacteria in turn develops resistance against these drugs and therefore causing treatment failure in the patients[8,9].

The nosocomial infections that are acquired are mostly through exposure to the hands of health care professionals after they have been temporarily colonized with staphylococci from contact with an infected patient or from their own reservoir. Because of all this, the bacteria are able to survive and bring harm to the patient, resulting in prolonging the stay in the hospital and even causing death of a patient[10].

Because of the increased use of the intravascular devices in the hospital setup, the threat of developing methicillin resistant *Staphylococcus aureus* has amplified[11]. Methicillin resistance in S. aureus not only means inadequate effectiveness of antibiotic treatment, but also showed prolonged hospital stay, financial burden on the patient and the state, and higher morbidity and mortality rates. A study showed that 20% of individuals are persistently colonized in the nose with S. aureus whereas there is also intermittent colonization of 30%-60% in some cases and 20% having no nasal carriage at all. Other than anterior nares, numerous other sites are also involved which includes the gastrointestinal tract, groin and axillae[12]. This is usually seen where there is prolonged insertion of a catheter especially in patients who are in dialysis, or they are admitted for surgery. S. aureus infections are generally occurring due to the strains, which are colonizing the patients at some point in time. In one study done in the patients suffering from bacteremia, blood isolates were found to be identical to the isolates in the nose in 82% of patients[13,14]. Diagnosis can be easily made by culturing the microorganism from the site of infection. According to the standards given to us by CLSI, we determined the methicillin resistance in *Staphylococcus aureus* when the diameter of zone of inhibition was equal or less than 21 mm to a cefoxitin disc diffusion test[15,16].

METHODS

A cross sectional study was done in the Department of Microbiology of Basic Medical Sciences Institute (BMSI) JPMC, Karachi from January 2015 to December 2015 after approval from the Institutional Ethical Committee. Sample size was calculated by Open Epi software according to the reference for the sample size[17]. Samples of pus from the patients' surgical site wounds, skin lesions and abscesses were collected from all the concerned departments of the hospital, especially medical wards, medical ICUs, surgical wards and surgical ICUs of Jinnah Post graduate Medical Centre. Patient's consent was given the utmost importance. Collection of pus was done from all skin lesion, any deep wounds, skin abscesses, suppurative postoperative wounds of the patients and samples were transported then to Basic Medical Sciences Institute (BMSI), JPMC for identification and processing.

The criteria to label the patient having HA-MRSA was determined as described previously[18,19]. Briefly, patient had one of the risk factors which includes the patient was hospitalized in the ward for more than 48 hours, admitted in ICU setup for more than 48 hours, hospitalized in the preceding year, had surgery in the preceding year, dialysis occurring in the previous year and the patient had a cannula or indwelling catheter in the previous year[18,19]. Patients who had started taking antibiotics or having infection other than *Staphylococcus aureus* were excluded from this study.

Only after taking the consent of the patients and explaining them about the importance of the process, the pus samples were collected. A sterile cotton-wool swab was used, and the sample was carefully taken from the site of infection having pus. It was then inoculated very carefully onto Blood agar medium, MacConkey agar medium and Mannitol Salt Agar to detect the presence of Staphylococcus aureus. A temperature to incubate the plates of culture medium was set to 37°C in the incubator and it was given time of 24 to 48 hours so that the organism grow on the culture media plates in the incubator. Isolation of *Staphylococcus aureus* was done from the culture obtained. Smears were stained and identified based on Gram staining procedure. The morphology of the colony and the bacteria was carefully observed. Catalase tests and coagulase test including the slide and the tube methods were done in addition to Mannitol fermentation tests.

After comparing the bacterial suspension to 0.5 McFarland standard suspensions, the susceptibility testing for antibiotics was done. Kirby-Bauer disc diffusion method was used to detect Methicillin resistance, by using cefoxitin (30µg) disk according to Clinical and Laboratory Standards institutes (CLSI) guidelines[20]. Isolates of HA-MRSA were then tested for sensitivity with TMP/SMX, tetracycline, erythromycin, clindamycin, ciprofloxacin, rifampicin, gentamicin, and linezolid by disk diffusion test by making a lawn culture of bacteria in Muller Hinton Agar (MHA) plates. Inducible clindamycin resistance was detected by D-Test. MIC was determined for vancomycin by E-Test according to the manufacturer's instruction. The data was analyzed by SPSS version 16. A descriptive analysis of continuous and categorical variables was performed.

Data on continuous variables was presented as mean +/- SD and data on categorical variables was presented as proportions.

RESULTS

The samples were collected from surgical sites, abscess, ulcers, other wound infections, catheters and cannulae. Out of 800 samples of pus collected from all wards of the tertiary care hospital, *Staphylococcus aureus* was detected in 402(50.2%) samples. Out of these samples containing Staphylococcus aureus, 149(37.06%) samples of pus had MRSA. From these MRSA samples, 106 (71.14%) belonged to HA-MRSA as per the criteria discussed above (Figure 1).

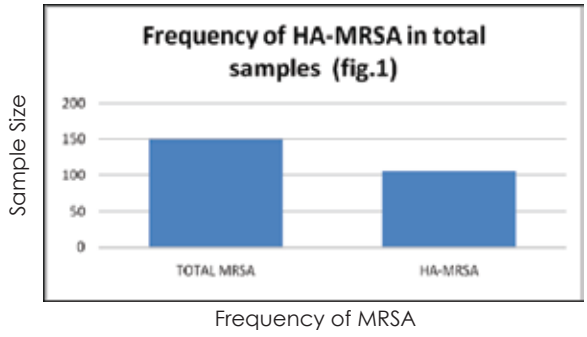

Figure 1: Frequency of HA-MRSA in total samples of MRSA.

In this study, we found that the 64 males (60.38%) had their pus positive for HA-MRSA, which was more as compared to 42 females (39.62%). The mean age of patients was found to be infected with HA-MRSA was 57.7 (±9.04) years. In a total of 106 HA-MRSA isolates, 91(85.8%) were sensitive to TMP/SMX, 98(92.5%) to rifampicin, 12(11.6%) to gentamicin, 85(80.2%) to tetracycline, 11(10.4%) to erythromycin, 37(34.9%) to clindamycin, 20(18.9%) to ciprofloxacin, 106 (100%) were sensitive to both vancomycin and linezolid antibiotics (Figure 2).

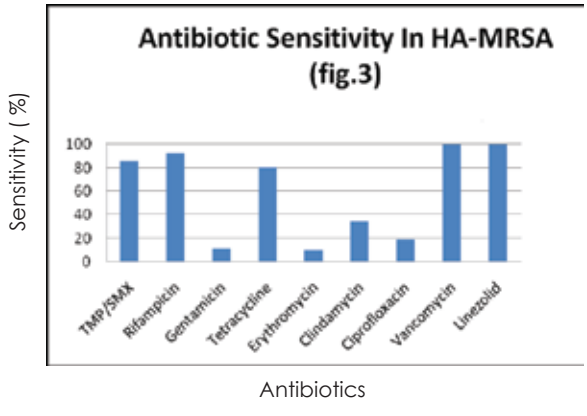

Figure 2: Antibiotic sensitivity pattern in HA-MRSA.

When D-Test was done to detect inducible resistance in clindamycin using erythromycin, sensitivity decreased to 22(23.32%). This was a double disc diffusion test showing a blunted zone of inhibition on the side, which faced the erythromycin disc (Figure 3).

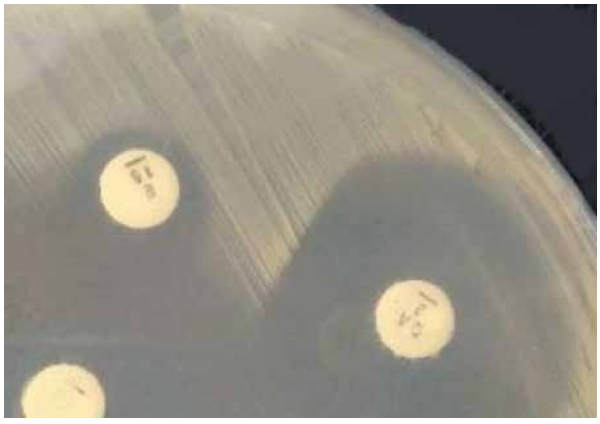

Figure 3: HA-MRSA on Muller Hinton Agar showing positive for D-test.

DISCUSSION

HA-MRSA is a recognized health care problem of the public admitted in the hospitals for a prolonged period. According to this study, nearly one thirds of the patients admitted in the wards acquired the resistant form of Staphylococcus aureus. This proved that the trend of acquiring HA-MRSA had not lessened despite the fact that there is a continuous spread of awareness through numerous programs and CMEs arranged for health care professionals and common people regarding hazards of self medication , over –the- counter availability of antibiotics and injudicious use of antibiotics[21,22]. According to the study done, out of all MRSA identified in the pus samples, it showed that patients having a purulent infection of the surgical wound with MRSA, admitted in a hospital setting were infected by Hospital Acquired-Methicillin Resistant *Staphylococcus aureus* (HA-MRSA). Moreover, that made up to 71.14%, the figures are close to a research done in Egypt, which showed 76.6% of patients infected by the same microorganism[23].

HA-MRSA was seen in older patients, with having a mean age of 57.7 years as shown in results which may be due to greater numbers of patients being admitted in ICUs and hospital wards, more likely to have chronic disease and unable to counter infections due to old age and weakness. Males infected with HA-MRSA made a greater proportion in the hospital (67.66%), the reason that may be due to more exposure in overcrowded places and hospital environment, therefore resulting in more skin and soft tissue infections as shown in the results[24].

Although antibiotic susceptibility testing is important for appropriate treatment of infections and cure of the disease, and for local susceptibility surveillance for the benefit of the community. One

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of the disadvantages of being infected by HA-MRSA is that it prolongs the hospital stay[25] and hence increases the financial burden on the class of patients with low socioeconomic status due to the use of more expensive antibiotics. Major part of the pus samples that came to our institute belonged to the surgical wards and Medical and surgical ICUs[26]. Our study also reported that the organism was sensitive to most of the drugs like trimethoprim/sulfamethoxazole and vancomycin. Less expensive agents should be tried keeping in mind the socioeconomic status of the patient coming to a tertiary care hospital with MRSA especially when it is still showing sensitivity to it.

Therefore these antibiotics can be used in combination therapy keeping in mind the social and economic status of the patients, as also seen in a similar study done in another part of the our continent[27]. Rifampicin can also be used to decrease nasal carriage of Staphylococcus aureus. However, clindamycin sensitivity should be confirmed after performing a D-Test with erythromycin as one of a previous study showed a 72% inducible resistance to this drug[28].This is important to get true susceptibility results for clindamycin and thus it prevents therapeutic failure. False susceptibility results for clindamycin may be obtained if isolates especially HA-MRSA are not tested for inducible resistance through D-testing by erythromycin. One relieving fact shown in our study was that all isolates were sensitive to linezolid in addition to vancomycin confirming an alternative for treating HA-MRSA infection and therefore therapeutic success, a trend seen in other part of the subcontinent also[29].

We are conducting numerous awareness programs and CMEs regarding injudicious use of antibiotics in clinical setup, more work is required. Spreading of awareness in healthcare personnel and attendants of the patients, regarding handling of the patients with a resistant microorganism and imparting the concept of isolation in them is crucial. Following these standards, it will reduce the cost of management and hospital stay in a developing country like us.

CONCLUSION

The present study thus concludes that a nosocomial infection, particularly HA-MRSA, is a significant problem, male patients were more than the female patients were. Patients were found sensitive to both vancomycin and linezolid in our health care setup.

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CONFLICT OF INTEREST

There is no conflict of interest among the authors.

ETHICS APPROVAL

The ethical approval was sort from BMSI, JPMC Institutional Board Committee.

PATIENT CONSENT

Verbal and written consent was obtained from all patients.

AUTHORS' CONTRIBUTIONS

SR is the corresponding author and conducted the study; NJ and SS helped in the acquisition of data SP did the analysis of data, and AS completed the final version for publication, FS guided and helped in the write-up, SMH was the supervisor of the study.

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