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Detection of Methicillin Resistant *Staphylococcus aureus* (MRSA) in Chronic Osteomyelitis

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Methicillin Resistant *Staphylococcus aureus* (MRSA) is an important hospital pathogen, incidence of Methicillin Resistant *Staphylococcus aureus* is on rise. The present study was performed to know the incidence of MRSA in chronic, osteomyelitis patients admitted in a tertiary care hospital. The bacteria causing chronic osteomyelitis were isolated using standard laboratory techniques. MRSA was identified using oxacillin (1ug) disc by modified Kirby Bauer disc diffusion method. The antibiotic sensitivity testing of *S. aureus* isolates was done to other antibiotics also. The proportion of MSRA among *S.aureus* isolates was 63.04%. The study shows increasing incidence of MRSA in chronic osteomyelitis as these strains may be hospital acquired or community acquired. All the MRSA were sensitive to Vancomycin. The present study also shows that these MRSA are less sensitive to other antibiotics.

Key words: Methicillin Resistant *Staphylococcus aureus* (MRSA), Chronic osteomyelitis, Oxacillin, Vancomycin.

S.aureus has been reported as a major cause of community and hospital acquired infections. Methicillin resistant *Staphylococcus aureus* (MRSA) strains are now worldwide after the introduction of methicillin. The rapid spread of bacteria resistant to antimicrobial a global phenomenon is perceived to be higher in developing countries attributed mainly to misuse of antibiotics.

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The organism has a differential ability to spread and cause outbreaks in hospitals. Infections caused by *S. aureus* used to respond to lactam and related groups of antibiotics[1]. However due to development of methicillin resistance among *Staphylococcus aureus* isolates elimination of MRSA is difficult. Indiscriminate use of multiple antibiotics, prolonged hospital stay, carriage of MRSA in nose of health care providers has led to increased incidence of MRSA. Chronic osteomyelitis is caused by many bacteria, most commonly *S. aureus* and other bacteria[2]. It is usually the sequelae of trauma, crush injuries and post-operative wound infections. Resistant causal organisms are frequently isolated. Isolation of the causal organisms and performance of susceptibility studies are critical in the selection of antimicrobial therapy[3]. The present study was performed to know the incidence of MRSA in chronic osteomyelitis patients admitted in a tertiary care hospital.

MATERIALS AND METHODS

A total of 100 clinically diagnosed chronic osteomyelitis patients were studied. One hundred clinical specimens (deeper discharge, dead bone, and exudating granule) were processed for both aerobic and anaerobic bacteriological examination. A preliminary gram staining was performed to know the likely organism present. Bacteria causing chronic osteomyelitis were isolated and identified by growing on nutrient agar, Mac conkey agar and 5% Sheep blood agar. Organisms were identified by standard laboratory techniques. *S.aureus* was identified based on colony morphology, catalase test, slide and tube coagulase test, Hugh and Leifson's Oxidative – Fermentative test and Mannitol sugar fermentation test. Antibiotic sensitivity testing was performed for all isolates by modified Kirby Bauer disc diffusion method on Mueller Hinton agar incubated at 35^0C for 24 hours. The antibiotic discs used were Ampicillin(10µg), Cefotaxime(30µg), Cotrimoxazole (1.25µg/23.75µg), Ceftazidime (30µg), Amikacin(30µg), Imipenem (10µg), Gentamicin (10µg), Ciprofloxacin (5µg), Erythromycin (5µg), Cephalexin(30µg), Cefuroxime (30µg) and Cefipime (30µg). Methicillin resistance among *S.aureus* isolates was detected by using Oxacillin 1ug disc obtained from Hi-media laboratories Pvt. Ltd on Mueller Hinton agar incubated at 30^0C for 24 hours. A zone of inhibition of 10mm or less or any discernible growth within zone of inhibition was indicative of methicillin resistance, zone of 13 mm or more without any growth in zone of inhibition was indicative of Methicillin Sensitive *Staphylococcus aureus*. Methicillin resistant strains were also confirmed by oxacillin screening agar using 6ug/ml of oxacillin in Mueller Hinton agar with 4% sodium chloride. Plates were incubated at 30^0C and strains showing growth on this medium were taken as Methicillin Resistant *Staphylococcus aureus*. All the MRSA strains were tested for Vancomycin (30ug) sensitivity. *S.aureus* ATCC 25923 was used as a standard control strain [4,5].

RESULTS

A total of 100 clinical specimens (deeper discharge, dead bone, granules) from 100 chronic osteomyelitis cases were taken. Eighty two (82%) specimens showed bacterial growth. A total of 110 organisms were isolated from chronic osteomyelitis cases. Among these isolates 46(41.82%) were *Staphylococcus aureus* either as single isolate or part of mixed bacterial flora causing chronic osteomyelitis. Table 1 shows the details of different organisms isolated. Out of 110 organisms isolated, aerobes were 101 (91.82%) and anaerobes 9 (8.18%). Among aerobes, *Staphylococcus aureus* 46 (41.82%), followed by *Pseudomonas spp.* 24

Table 1. Showing different organisms isolated

Organisms	Number of organisms	Percentage (%)
Aerobes (n = 101)		
1. *Staphylococcus aureus*	46	41.82
Pseudomonas spp.	24	21.82
2. *P. aeruginosa* → 21		
3. *P. fluorescens* → 2		
4. Non-pigment producing *Pseudomonas* → 1		
5. *Escherichia coli*	12	10.91
6. *Staphylococcus epidermidis*	11	10.00
7. *Klebsiella pneumonia*	4	3.64
8. *Enterococcus faecalis*	3	2.72
9. *Proteus mirabilis*	1	0.91
Anaerobes (n = 9)		
10. *Peptostreptococcus spp.*	5	4.54
11. *Bacteroides spp.*	4	3.64
Total	110	100

(21.82%), *Escherichia coli* 12 (10.91%), *S. epidermidis* 11 (10%), *Klebsiella pneumoniae* 4 (3.64%), *E. faecalis* 3 (2.72%) and *P. mirabilis* 1 (0.91%) were isolated. Among anaerobes, *Peptostreptococcus* spp. 5 (4.54%) and *Bacteroides spp.* 4 (3.64%) were isolated.

Out of 46 *Staphylococcus aureus* isolated, 17 (36.96%) were Methicillin sensitive *Staphylococcus aureus* and 29 (63.04%) were Methicillin Resistant *Staphylococcus aureus*. All the MRSA were Sensitive to Vancomycin (100%).

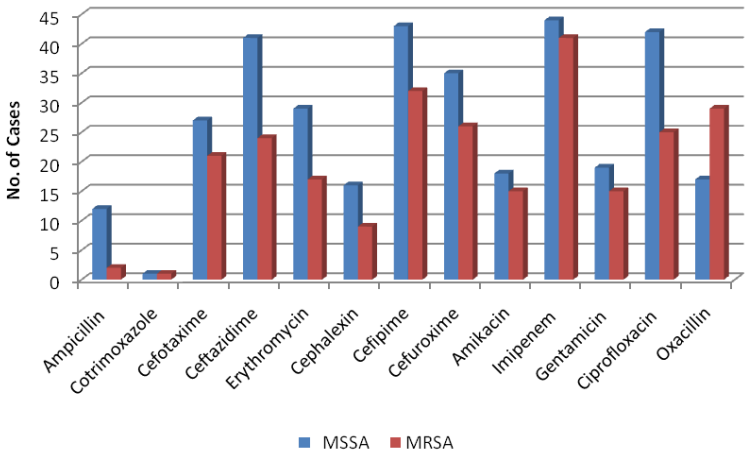

Fig. 1. Antibiotic sensitivity pattern of Methicillin Sensitive *Staphylococcus aureus* (MSSA) and Methicillin Resistant *Staphylococcus aureus* (MRSA) to various antibiotics.

DISCUSSION

Chronic osteomyelitis is a long standing bone infection difficult to treat conservatively. The infection of bone with multi drug resistant organisms is becoming more common. Invasion of bone with organisms like methicillin resistant *S. aureus* either in hospital or community is difficult to treat with commonly available antibiotics. MRSA has become a well established hospital or community acquired strain. Chronic osteomyelitis is one of the risk factors for easily acquiring MRSA either in hospital or community because of open discharging sinus or long stay in hospital for treatment. Majority of the organisms isolated in the current study were Gram positive cocci (*S.aureus*), Enterobactericeae and Non-fermenters (*Pseudomonas* spp.*)* which is in correlation with other studies[3]. A total of 46(41.82%) *S.aureus* out of 110 organisms was isolated. Out of 46 *S.aureus* isolated 17(36.96%) were methicillin sensitive and 29(63.04%) methicillin resistant. Present study shows a raising percentage of MRSA (63.04 %) isolation which is comparable to other studies reporting a higher incidence of MRSA more than 50% (Vidhani *et al.*,[6] 51.8%, Majumder *et al.*,[7] 52.9%, Anurpba *et al.*,[8] 54.85%). However there is insufficient recent data about MRSA detected in chronic osteomyelitis. All these studies have shown general prevalence of MRSA in community and hospital. Some earlier studies showed a less incidence of MRSA compared to present study (Chakravarhty *et al.*,[9] 6.9%, Udayashankar *et al.*,[10] 20%, Umachaudhary *et al.*,[11] 23.8%, Pulimod *et al.*,[12] 24%, Hanumanthappa *et al.*,[13] 43%). MRSA was not so commonly isolated in the past but now definitely there is a rise of MRSA. The reasons may be due to failure to identify MRSA early and treating without antibiotic sensitivity report, rapid spread of MRSA in community and hospital, could be due to incomplete treatment, indiscriminate use of antibiotics, improper aseptic conditions in hospital atmosphere[14]. Bone is very sensitive tissue which will get infected easily and also the pathogenicity factors of *S. aureus* help the organism to adhere firmly to connective tissue (glycocalyx) to establish the infection[15,16]. There was difference in the antibiotic sensitivity pattern

of MRSA and MSSA for other antibiotics also. MSSA were more sensitive to parenteral antibiotics like cephalosporins (ceftazidime 89.13%, cefuroxime 76.08%, cefipime 93.47%),Quinolones (ciprofloxacin91.30%) and imipenem (95.65%) compared to MRSA less sensitive to these antibiotics but MRSA were also sensitive to imipenem (89.13%) this was comparable with observation of Vidhani *et al.*,[6] who have reported a higher degree of resistance to all other antibiotics by MRSA compared to MSSA.

CONCLUSION

Chronic osteomyelitis is the common form of osteomyelitis in adults and is usually the sequelae of trauma. Isolation of causal organism and performance of sensitivity studies are critical in the selection of antimicrobial agents. Resistant causative organisms are frequently isolated. Carefully selected antibiotic therapy guided by culture and sensitivity is an effective treatment modality. This will prevent development of drug resistance and indiscriminate use of antibiotics. Once MRSA colonised in hospitals, hospital staff and patients it is difficult to eliminate. Raising incidence of MRSA in tertiary hospitals highlights strict aseptic measures and hospital infection control measures to prevent and control of MRSA rapid spread.

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