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A study of prevalence of staphylococcus aureus and its antibiotic susceptibility pattern in 100 osteomyelitis patients in a tertiary care teaching hospital

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Abstract

Background: Osteomyelitis is an important cause of morbidity in patients with bone infections in developing countries like India. Gram positive bacteria especially *Staphylococcus aureus*, classically causes this disease, but Gram negative bacteria have grown in importance as causative agents. Widespread use of antibiotics has however altered the aetiological pattern of infections and antibiotic susceptibility pattern. Therefore, the treatment is becoming increasingly troublesome due to rise in drug resistant isolates.

Objective: This study was therefore undertaken to determine the bacteriological profile of osteomyelitis and also to ascertain the antibiotic susceptibility pattern of the isolates.

Materials and Methods: In the present study, 100 pus samples from osteomyelitis patients were taken and the specimens were inoculated onto Nutrient, blood and MacConkey agar plates. The plates were incubated at 37 °C for 18 – 24 hours in an incubator. Culture isolates were identified by colony characteristics, Gram's staining, motility and biochemical reactions. Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method using CLSI guidelines. MRSA detection was done by cefoxitin disc diffusion method.

Results: The highest incidence of osteomyelitis was observed in the age group of 16-30 years (34%) followed by 31-45 years (31%). Out of 100 cases, 76 (76%) were males and 24 (24%) were females with male to female ratio of 3.16:1. Trauma due to road side accidents (45%) accounted for maximum no. of cases. The commonest organism isolated was *Staphylococcus aureus* – 32 (35.56%) followed by *Pseudomonas aeruginosa* -17 (18.89%) as the second most common organism. Out of 32 *Staph. aureus* isolates, 13 (41.93%) isolates were found to be MRSA and all the MRSA isolates were (100%) resistant to ampicillin, amoxycylav and cefotaxime.

Conclusion : Our study will guide the clinicians in choosing appropriate antibiotics, which not only contribute to better treatment, but the judicious use of such antibiotics will also help in preventing emergence of resistance to the drugs, which are still sensitive.

Keywords: Osteomyelitis, *Staphylococcus aureus*, antibiotic susceptibility, Methicillin Resistant *Staphylococcus aureus*.

Introduction

Osteomyelitis or bone infection is an inflammation of bone caused by an infectious organism such as bacteria. However, fungi, parasites or viruses can also cause osteomyelitis^[1]. It affects both adults and children. Patients with certain conditions such as intravenous drug abusers, decubitus ulcers, surgery, trauma, immunosuppression, diabetes mellitus, smoking, malnutrition, malignancy and extremes of age are at increased risk of osteomyelitis^[2]. Osteomyelitis can occur in a variety of bones in different areas of the body. The bones commonly involved in children are long bones such as femur, tibia, humerus and radius. In adults, it usually affects the vertebral column, in particular the lumbar spine, sacrum and the pelvis^[1].

Staphylococcus aureus is the most commonly isolated pathogen for both acute and chronic osteomyelitis in all age groups^[2]. There has been increase in methicillin- resistant *Staph. aureus* (MRSA) in recent years due to rapid development of antimicrobial resistance and expression of virulence factors, regardless of the patient's immune status.

Coagulase-negative staphylococci are often seen in association with foreign bodies, such as prosthetic joints [3]. Also, Gram negative bacteria, such as Pseudomonas aeruginosa and Enterobacteriaceae have grown in importance as causative agents due to the rising number of high – energy traumas associated with open fractures [4].

Widespread use of antibiotics has however altered the aetiological pattern of infections and antibiotic susceptibility pattern. Hence continuous monitoring of susceptibility pattern needs to be carried out so as to detect the true burden of antibiotic resistance among organisms and prevent their further emergence by judicious use of drugs [5].

This study was therefore undertaken to determine the bacteriological profile of osteomyelitis and also to ascertain the antibiotic susceptibility pattern of the isolates that will help the clinician in deciding the treatment regime for the patients from the beginning of the treatment and avoid indiscriminate use of antibiotics.

Materials and Methods

In the present study, 100 pus samples from osteomyelitis patients attending the outdoor and indoor units of orthopaedics department, Rajindra hospital, Patiala were taken from July 2016 to Sep 2017. Pus was collected aseptically from the depth of the wound with a sterile syringe or swabs prior to administration of antimicrobial agents. The specimens were examined for colour, odour and consistency. Smears were made from pus and stained with Gram’s staining to determine the morphology of organisms. For isolation of organisms, the specimens were inoculated onto Nutrient, blood and MacConkey agar plates. The plates were incubated at 37 °C for 18 – 24 hours in an incubator. Culture isolates were identified by colony characteristics, Gram’s staining, motility and biochemical reactions and any other special test for confirmation of particular organism.

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method using CLSI guidelines [6]. The antibiotic discs were obtained from Hi – Media Laboratories Pvt. Ltd, Mumbai.

The various antibiotics used for Gram positive organisms were:- ampicillin, gentamicin, amikacin, amoxyclav, ciprofloxacin, cotrimoxazole, cefoxitin, cefotaxime, erythromycin, clindamycin, linezolid and vancomycin. The antibiotics used for Gram negative organisms were:- ampicillin, gentamicin, amikacin, amoxyclav, ciprofloxacin, cotrimoxazole, cefotaxime, ceftazidime, ceftazidime-clav, piperacillin/tazobactam and imipenem.

CLSI recommends cefoxitin disc diffusion method for MRSA detection. It was performed by using cefoxitin disc on Mueller-Hinton medium and plates were incubated at 33-35 °C for 16-18 hours and zone diameter was measured in the presence of reflected light.

Interpretative Criteria for Cefoxitin Disc Diffusion Test – in mm		
	Susceptible	Resistant
S. aureus	≥ 22 mm	≤ 21 mm

Results

The highest incidence of osteomyelitis was observed in the age group of 16-30 years (34%) followed by 31-45 years (31%), > 46 years (24%) and 0-15 years (11%). The age range was found to be 5-75 years while the median age was

observed as 34 years. Out of 100 cases, 76 (76%) were males and 24 (24%) were females with male to female ratio of 3.16:1.

The predisposing factors for osteomyelitis are listed in table 1. Trauma due to road side accidents (45%) accounted for maximum no. of cases. Other factors responsible were postoperative infections (21%), orthopaedic implants (17%), haematogenous (11%), diabetes mellitus (4%) and others (2%)

Table 1: Predisposing factors for osteomyelitis

Sr. No.	Pre disposing factors	No. of isolates	Percentage
1	Trauma due to road side accidents	45	45%
2	Post-operative infections	21	21%
3	Orthopaedic implants	17	17%
4	Haematogenous	11	11%
5	Diabetes mellitus	4	4%
6	Others	2	2%
Total		100	100%

Table 2 shows the order of involvement of various bones. Tibia was the most commonly involved bone in 36% cases, followed by femur (32%), bones of foot (9%), humerus (6%), radius (5%), bones of hand (4%), fibula and ulna (3% each) and clavicle (2%).

Table 2: Bones involved in osteomyelitis

Sr. No.	Bones involved	No. of isolates	Percentage
1	Tibia	36	36%
2	Femur	32	32%
3	Bones of foot	9	9%
4	Humerus	6	6%
5	Radius	5	5%
6	Bones of hand	4	4%
7	Fibula	3	3%
8	Ulna	3	3%
9	Clavicle	2	2%
Total		100	100%

In a total of 100 cultures put up, growth of organisms was obtained in 84 (84%) cases and 16 (16%) cases were sterile. Out of 84 positive cultures, single organism was obtained in 78 (92.86%) cases and two organisms were isolated in 6 (7.14%) cases accounting for a total of 90 isolates (Figure 1). The commonest organism isolated was Staphylococcus aureus – 32 (35.56%) followed by Pseudomonas aeruginosa -17 (18.89%) as the second most common organism. (Figure 2)

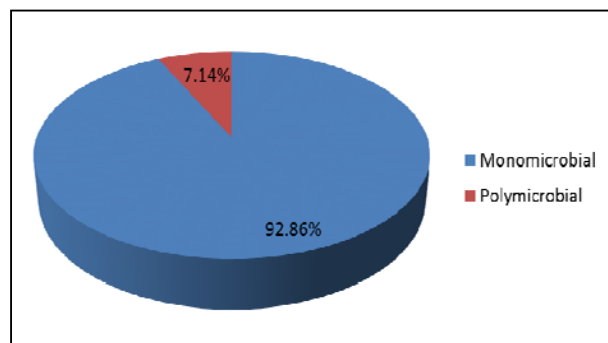


Fig 1: Type of Isolate found in positive culture cases

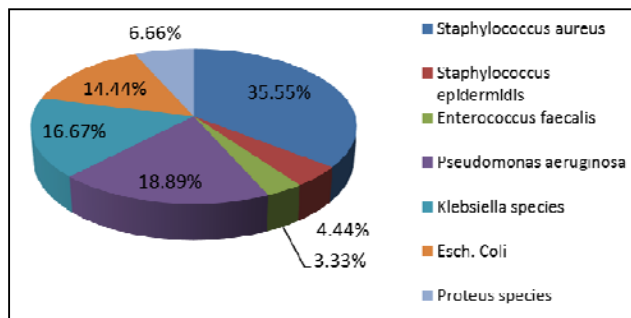


Fig 2: Percentage of various pathogens in positive culture cases

Table 3 shows the antibiotic susceptibility pattern of Gram positive isolates. Staph.aureus showed 100% sensitivity to vancomycin followed by linezolid (96.87%), clindamycin (78.12%) and amikacin (71.87%). Staph. epidermidis was found to be 100% sensitive to vancomycin followed by 75%

sensitivity to each of linezolid and amikacin. E. faecalis showed resistance to all other drugs except vancomycin, linezolid and amikacin. Table 4 shows the antibiotic susceptibility pattern of Gram negative isolates.

Table 3: Antibiotic susceptibility pattern of Gram positive isolates

Sr. No.	Antibiotic	Staph. aureus (n=32)	Staph. epidermidis (n=4)	E. faecalis (n=3)
1	Ampicillin	5 (15.62%)	1 (25%)	0 (0%)
2	Gentamicin	16 (50%)	1 (25%)	0 (0%)
3	Amikacin	23 (71.87%)	3 (75%)	1 (33.33%)
4	Amoxyclav	11 (34.37%)	2 (50%)	0 (0%)
5	Ciprofloxacin	10 (31.25%)	1 (25%)	0 (0%)
6	Cotrimoxazole	16 (50%)	2 (50%)	0 (0%)
7	Cefoxitin	19 (59.37%)	2 (50%)	0 (0%)
8	Cefotaxime	13 (40.62%)	3 (75%)	0 (0%)
9	Erythromycin	15 (46.87%)	2 (50%)	0 (0%)
10	Clindamycin	25 (78.12%)	2 (50%)	0 (0%)
11	Linezolid	31 (96.87%)	3 (75%)	3 (100%)
12	Vancomycin	32 (100%)	4 (100%)	3 (100%)

Table 4: Antibiotic susceptibility pattern of Gram negative isolates

Sr. No.	Antibiotic	P. aeruginosa (n=17)		Klebsiella spp. (n=15)		Esch. coli (n=13)		Proteus spp. (n=6)	
1	Ampicillin	0	0%	0	0%	2	15.38%	0	0%
2	Gentamicin	4	23.53%	11	73.33%	4	30.76%	2	33.33%
3	Amikacin	9	52.94%	12	80%	11	84.61%	4	66.66%
4	Amoxyclav	7	41.18%	0	0%	4	30.76%	0	0%
5	Ciprofloxacin	7	41.18%	7	46.66%	8	61.54%	0	0%
6	Cotrimoxazole	4	23.53%	5	33.33%	5	38.46%	0	0%
7	Cefotaxime	4	23.53%	7	46.66%	4	30.76%	0	0%
8	Ceftazidime	3	17.64%	2	13.33%	5	38.46%	0	0%
9	Ceftazidime-clav	4	23.53%	3	20%	6	46.15%	0	0%
10	Piperacillin-tazobactam	9	52.94%	4	26.66%	12	92.30%	5	83.33%
11	Imipenem	12	70.58%	12	80%	13	100%	5	83.33%

Out of 32 Staph. aureus isolates, 13 (41.93%) isolates were found to be MRSA and 19 (59.37%) isolates obtained were MSSA. The antibiotic sensitivity pattern of MRSA was observed as follows - vancomycin (100%), linezolid (100%),

clindamycin (61.54%), amikacin (61.54%), cotrimoxazole (38.46%), erythromycin (30.77%), gentamicin (7.70%), ciprofloxacin (7.69%). All the MRSA isolates were (100%) resistant to ampicillin, amoxyclav and cefotaxime.

Table 5: Antibiotic susceptibility pattern of MRSA and MSSA isolates

Sr. No.	Antibiotic		MRSA (n=13)		MSSA (n=19)		Chi Square X ²	p value
			Number	Percent	Number	Per cent		
1	Ampicillin	Sensitive	0	0%	5	26.31%	4.055	0.044 (S)
		Resistant	13	100%	14	73.69%		
2	Gentamicin	Sensitive	2	15.38%	14	73.69%	10.494	0.001 (S)
		Resistant	11	84.62%	5	26.31%		
3	Amikacin	Sensitive	8	61.54%	15	78.95%	1.157	0.282 (NS)
		Resistant	5	38.46%	4	21.05%		
4	Amoxyclav	Sensitive	0	0%	11	57.90%	11.469	0.001 (S)
		Resistant	13	100%	8	42.10%		
5	Ciprofloxacin	Sensitive	1	7.70%	9	47.37%	5.656	0.017 (S)
		Resistant	12	92.30%	10	52.63%		
6	Cotrimoxazole	Sensitive	5	38.46%	11	57.90%	1.166	0.280 (NS)
		Resistant	8	61.54%	8	42.10%		
7	Cefoxitin	Sensitive	0	0%	19	100%	32.00	<0.001 (S)
		Resistant	13	100%	0	0%		
8	Cefotaxime	Sensitive	0	0%	13	68.42%	14.981	<0.001 (S)
		Resistant	13	100%	6	31.58%		
9	Erythromycin	Sensitive	4	30.77%	11	57.90%	2.281	0.131 (NS)
		Resistant	9	69.23%	8	42.10%		
10	Clindamycin	Sensitive	8	61.54%	17	89.47%	3.525	0.060 (NS)
		Resistant	5	38.46%	2	10.53%		
11	Linezolid	Sensitive	13	100%	18	94.74%	0.706	0.401 (NS)
		Resistant	0	0%	1	5.26%		
12	Vancomycin	Sensitive	13	100%	19	100%	NA	
		Resistant	0	0%	0	0%		

NS (Not Significant) $p > 0.05$; S (Significant) $p < 0.05$ at $df = 1$, NA = not applicable

Discussion

The highest incidence of osteomyelitis was observed in the age group of 16-30 years (34%) followed by 31-45 years (31%), > 46 years (24%) and 0-15 years (11%) Similar observations were made in a study by Kaur J *et al.* [5], who showed that occurrence of osteomyelitis was higher in 16-30 (36%) and 31-45 (34%) age groups than in children and elderly age group and by Qureshi M *et al.* [7] who showed that maximum cases of osteomyelitis were found in age group of 15-30 years (32.7%) followed by 31-50 age group (31.5%).

In our study, osteomyelitis cases were more common in males (76%) as compared to females (24%) with a male: female ratio of 3.16:1. Similar results were reported by Kumar A *et al.* [8], Jiang N *et al.* [9] and Singh A *et al.* [10] who showed male: female ratio to be 2.72:1, 3.53:1 and 2.7:1 respectively.

In the current study, the most common predisposing factor leading to osteomyelitis was trauma due to road side accidents accounting for 45% cases followed by post-operative infections (21%), orthopaedic implants (17%), haematogenous (11%), diabetes mellitus (4%) and others. This is in concordance with a study by Wadekar MD *et al.* [11] who concluded trauma (44%) as the commonest predisposing factor followed by post-operative infections (23%) and orthopaedic implants (23%) and also similar to the findings of Khatoun R *et al.* [12] according to whom the most common predisposing factor was trauma leading to open fractures (45.6%).

In the present study, it was found that tibia was the commonest bone affected by the osteomyelitis (36%), followed by femur (32%). However, our study differs from study of Wadekar MD *et al.* [11] which showed highest incidence of osteomyelitis in femur (48%) followed by tibia (23%).

Out of 100 pus samples processed in the present study, 84% were culture positive and 16% were culture negative This finding is in concordance with studies by Veerana HD *et al.* [13] and Suguneshwari G *et al.* [14].

Out of 84 positive cultures, single organism was isolated in 78 (92.86%) cases and two organisms were isolated in 6 (7.14%) cases. These results are consistent with a study by Salim N *et al.* [15] which showed 87.27% monomicrobial growth and 12.72% polymicrobial growth. A similar study by Shah FA *et al.* [16] from Pakistan also reported 92% growth as monomicrobial and 8% as polymicrobial.

In the present study, among the Gram positive organisms, Staph.aureus was isolated from maximum no. of patients (35.55%) followed by Staph. epidermidis (4.44%) and Enterococcus faecalis (3.33%) which is similar to the studies of Kaur J *et al.* [5] who isolated Staph. aureus in maximum no. of cases - 43% followed by Staph. epidermidis in 4% cases. Sheehy SH *et al.* [17] in his prospective study on 166 patients from Oxford, UK also reported Staph. aureus as the most common isolate (32%) amongst a wide range of organisms.

Among the Gram negative organisms, the most commonly isolated pathogen was Pseudomonas aeruginosa (18.89%) followed by Klebsiella species (16.67%), Esch. coli (14.44%) and Proteus spp. (6.66%). However, our study differs from observations of Kumar A *et al.* [8] who reported maximum incidence in E. coli (12.1%) and Gopi A *et al.* [18] who isolated Klebsiellapneumoniae in maximum no. of cases as 11.4%.

In the present study, Staph.aureus showed maximum sensitivity to vancomycin (100%) followed by linezolid (96.87%), clindamycin (78.12%) and amikacin (71.87%). Our

findings are supported by Gopi A *et al.* [18] who reported 100% sensitivity to both vancomycin and linezolid followed by high degree of sensitivity to clindamycin (94%). Chakravarty S *et al.* [19] also reported similar sensitivity pattern with sensitivity to vancomycin, linezolid and amikacin as 100%, 95.7% and 78.3% respectively.

Our study results showed that out of 39 strains of Staph. aureus, 41.93% strains were MRSA and MSSA accounted for 59.37% of the cases. Our study correlates well to the study of Salim N *et al.* [15] who reported incidence of MRSA and MSSA being 38.88% and 61.11% respectively.

In the present study, majority of the MRSA strains were resistant to commonly used antibiotics but showed 100% sensitivity to both vancomycin and linezolid followed by sensitivity to clindamycin and amikacin in equal proportion (61.54% each). These findings are comparable to the study of Wadekar MD *et al.* [11] who also reported 100% sensitivity to both vancomycin and linezolid followed by 78.5% sensitivity to amikacin. Ali M *et al.* [20] in his study reported 100% sensitivity to vancomycin and 75% sensitivity to linezolid and high degree of resistance to commonly used antibiotics like ciprofloxacin and cefotaxime.

It is quite clear from the previous studies and the present study that MRSA strains are becoming alarming because of their increased resistance towards antibiotics – like amikacin and gentamicin, that leaves the clinicians with limited choice to use appropriate drug for the treatment of osteomyelitis.

Conclusion

Osteomyelitis is even today one of the most challenging bone and joint infections. Gram positive bacteria especially Staphylococcus aureus, classically causes this disease, but Gram negative bacteria have grown in importance as causative agents. Gram negative bacilli and methicilin resistant Staph. aureus has been found to be quite high among the organisms isolated from osteomyelitis patients.

Emerging multidrug resistant strains is of major concern as they pose challenge in the treatment of osteomyelitis. Multidrug resistance among MRSA strains has serious implications as far as treatment of MRSA infections is concerned.

This study highlights the importance of culture-directed antibiotic therapy for osteomyelitis and thus helps the orthopaedician in choosing appropriate antibiotics which not only contribute to better treatment but their judicious use will also help in preventing emergence of resistance to the antibiotics which are still sensitive.

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