ORIGINAL ARTICLE

Bacterial pathogens prevalent amongst orthopaedic patients in New Delhi

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ABSTRACT

Background: The problem of changes in pathogenic microbiological flora and the emergence of bacterial resistance have created major problems in the management of orthopaedic diseases and fractures. We, therefore, have conducted this study to find out the frequency of bacterial flora in relation to the different clinical syndromes and the antibiotic sensitivity pattern of various bacterial isolates and thus guide the empirical antimicrobial chemotherapy in orthopaedic wound infections. **Materials and Methods:** A retrospective study of the bacterial isolates of pus specimen collected from orthopaedic patients who had various clinical diagnoses was carried out at the Routine Laboratory of Department of Microbiology. The culture and antimicrobial susceptibility patterns were reviewed for the period 2007 through 2012. **Results:** During the six year study period from 1st Jan 2007 to 31st Dec 2012, our laboratory received a total of 1722 specimens of pus whose site of sample collection included open fractures, bed sores, surgical site infection (SSI), synovial fluid and pin tract site infections. Of these, 900 (52.26%) specimens showed culture positivity including 62 specimens yielding >1 organisms, 822 specimens (47.73%) did not show growth of any pathogenic organism after 48 hours of aerobic incubation. The isolation rate of gram positive and gram negative organisms from various clinical syndromes was roughly similar. Most of the gram positive organisms were sensitive to Cefazolin and most of the gram negative organisms to Amikacin. **Conclusions:** We recommend the combined use of Amikacin and Cefazolin as the first drugs of choice for empirical therapy in orthopaedic patients with wound infections.

Key words: Antimicrobial susceptibility, microbiology, orthopaedic

INTRODUCTION

The problem of changes in pathogenic microbiological flora and the emergence of bacterial resistance have created major problems in the management of orthopaedic diseases and fractures. Due to the use of implants for open reduction and internal fixation, which are foreign bodies to the body, orthopaedic trauma surgery is at grave risk of microbial contamination and infection. During the past few years, there has been remarkable improvement in the field of diagnosis of infection due to newer techniques and sophisticated tools, better health care systems, particularly in urban areas, increasing awareness in patients, and invention of newer, more effective, and less toxic antimicrobials for combating osteoarticular infections.

The following factors influence the epidemiology of musculoskeletal infection:

- The prevalence of the underlying risk factors for bacteraemia, trauma and instrumentation in the population.
- The geographical distribution of pathogens.
- Socio-economic factors.[1]

The source of an infecting organism may be one of the following:

- Endogenous i.e., from a patient's own flora, which at the time of admission may include the organism brought into hospital at admission;
- Exogenous i.e., from another patient or a member of the hospital staff or from the inanimate environment of the hospital, environmental, air, water, food and medication; used equipment/instrumentation, soiled linen and hospital waste;
- Contamination of wounds during the time of injury by dirt, soot, grease, etc.[2-5]

Despite advances in diagnostic technologies patients with orthopaedic wound infections are being given empirical antibiotic therapy. We, therefore, have conducted this study to find out the prevalence of different species of bacteria in relation to the different clinical syndromes and the antibiotic sensitivity pattern of various bacterial isolates to guide the empirical antimicrobial chemotherapy in orthopaedic wound infections.

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MATERIALS AND METHODS

A retrospective study of the bacterial isolates of pus specimen collected from orthopaedic patients who had various clinical diagnoses was carried out at the Routine Laboratory of Department of Microbiology, Maulana Azad Medical College, New Delhi. The culture and antimicrobial susceptibility patterns were reviewed for the period 2007 through 2012. Our laboratory receives pus specimens for culture and antibiotic susceptibility test from all departments of Lok Nayak Hospital, which is the associated teaching hospital of the college. Pus specimens were received along with the requisition slips in either a sterile container or a sterile swab and were inoculated onto 5% sheep blood agar and MacConkey agar media as well as Brain Heart Infusion (BHI) broth. The clinical diagnosis of each specimen received was also recorded. The plates were examined for the growth of bacteria after 24 hours of aerobic incubation of plates at 37°C. If no growth was observed on the plates, subcultures were made from the glucose broth onto 5% sheep blood agar and MacConkey agar, which were observed after overnight incubation. The colonies were identified by standard microbiological techniques. The antibiotic susceptibility patterns of pathogenic organisms were determined on the day of their isolation by the modified Stoke's disc diffusion method on Mueller Hinton agar comparing the zones of inhibition of the test strain with that of the control strain to define sensitivity or resistance to different antimicrobials.

All the confirmed *Staphylococcus aureus* strains were subsequently tested for Methicillin resistance based on Kirby-Bauer disk diffusion method using Cefoxitin discs (30 μg) obtained from Hi-Media Laboratories Pvt. Ltd. The isolates were considered Methicillin resistant (MRSA) if the zone of inhibition was less than 21 mm and Methicillin sensitive (MSSA) if it was ≥ 21 mm. The specimens showing growth of only coagulase-negative *Staphylococcus spp* (CoNS) were considered to be contaminated with skin commensals and were not processed further unless the specimen was a synovial fluid and there was a suspicion of prosthetic joint infections. The antibiotics tested against *Staphylococcus aureus* were Penicillin-G, Cephalexin, Cefazolin, Erythromycin, Clindamycin, Gentamicin, Amikacin, Vancomycin, Teicoplanin, Linezolid, Rifampicin and Chloramphenicol. The following antibiotics were used for Gram Negative bacilli: Cephalexin; Ceftriaxone; Cefotaxime; Amoxicillin; Ciprofloxacin; Gentamicin; Amikacin; Imipenem; Meropenem; Piperacillin-Tazobactam and the antibiotics tested against *Pseudomonas spp* were Gentamicin, Amikacin, Ciprofloxacin, Aztreonam, Ceftazidime, Piperacillin-Tazobactam, Imipenem, Meropenem, Netilmicin and Tobramycin. The tests were interpreted as Sensitive, Intermediate susceptible or Resistant in accordance with standard recommendation.[6]

RESULTS

During the six-year study period from 1st Jan 2007 to 31st Dec 2012, our laboratory received a total of 1722 specimens of pus whose site of sample collection included open fractures, deep bed sores involving bones, surgical site infection (SSI), synovial fluid and pin tract site infections. Of these, 900 (52.26%) specimens showed culture positivity including 62 specimens yielding more than one organisms i.e., a total of 962 isolates obtained. After 48 hours of aerobic incubation, 822 specimens (47.73%) did not show growth of any pathogenic organism. The age of the patients ranged from 1 year to 78 years with a mean age of 29 and a standard deviation of 15. The incidence of various microbes in relation to orthopaedic illnesses and procedures and the antibiotic susceptibility pattern of infecting pathogenic bacteria are detailed in the following self-explanatory tables [Tables 1-5].

DISCUSSION

The most common pathogens found in orthopaedic wound infections and fractures in our study are *Staphylococcus aureus*, *Klebsiella spp*, *Pseudomonas spp, Escherichia coli, Acinetobacter spp*,

Table 1: Incidence of various microbes (excluding CoNS and *Micrococci spp*) in relation to orthopaedic illnesses and procedures

Pathogen	Open fracture	Synovial fluid	Bed sore involving bone	SSI	Pin tract site infection	Total (%)
Staphylococcus aureus	21	30	64	75	102	292 (30.35)
Streptococcus pyogenes	0	03	0	08	02	13 (01.35)
Enterococcus spp	0	0	07	0	06	13 (01.35)
Escherichia coli	19	04	34	27	80	164 (17.04)
Klebsiella spp	46	16	36	33	57	188 (19.54)
Proteus spp	03	01	14	10	21	49 (05.09)
Pseudomonas spp	05	03	08	60	97	173 (17.98)
Acinetobacter spp	0	0	12	04	42	58 (06.02)
Citrobacter spp	0	0	01	03	04	08 (0.83)
Enterobacter spp	0	0	0	0	02	02 (0.20)
Providencia spp	0	0	01	0	01	02 (0.20)
Total (%)	94 (09.77)	57 (05.92)	177 (18.39)	220 (22.86)	414 (43.03)	962 (100)

Proteus spp in that order. *S. aureus* (30.35% of the total number of isolates) is the most common organism isolated from this study. About 10% to 30% of healthy people carry this organism, particularly in the anterior nares. Bed sheets, instruments and dressings have also been found to act as reservoirs. Bergqvist *et al*.[7] and Dan *et al*[8] found that 29.8% of hospitalized patients and 26.6% of hospital staff, respectively are carriers. *Klebsiella spp* remains the second most common pathogen (19.54% cases), especially in open fractures and patients with pin tract site infections. *Klebsiella spp* is a commensal of gut and as many orthopaedic patients are bedridden for prolonged periods, contamination of wounds, dressing, linen, clothes and even hands during perineal hygiene plays a major role in increasing chances of transmission of infection. This holds true even for *E. coli* which has also been isolated in a significant number (17.04) in our study. The third most common microbe recovered from our center proved to be *Pseudomonas spp* (17.98%), which are commonly found in pin tract site infections and SSIs. Agrawal *et al*.[9] and Dade and Hall[10] have documented that *Pseudomonas* can multiply on common objects in a hospital environment such as buckets used for soaking Plaster of Paris bandages, wood wool paddings, and Cheatle forceps.

Harvey Bernard[11] shows that in the last several decades, the pattern of infection has been changing and gram negative bacteria are becoming more and more common. In this study although *S. aureus* is shown to be the most common individual pathogen isolated, gram negative infections continue to be a major threat and their isolation rate as a whole (66.94% of total number of isolates) far outnumbers that of gram positive infections (33.05%). This finding is in accordance with that of Agrawal *et al*.[9] who reported the incidence of gram negative bacteria to be 74.8%. Our study also shows that MRSA (isolation of one strain for every 3 strains of MSSA isolated) has emerged as a significant threat among orthopaedic patients. A similar antibiotic susceptibility pattern showing approximately 100% sensitivity toward Vancomycin, Teicoplanin, Linezolid, Rifampicin and Chloramphenicol is observed for both MSSA and MRSA. However, there is a striking difference between sensitivity of MSSA (92.8%) and that

Table 2: Overall rate of isolation of gram positive (GPC) and gram negative (GNR) pathogens

Pathogens		No of cases isolated	Total (% of total isolates)
Gram Positive Cocci	MSSA	221	318 (33.05)
	MRSA	71	
	Streptococcus spp	13	
	Enterococcus spp	13	
Gram Negative Rods	*Klebsiella spp*	188	644 (66.94)
	Escherichia coli	164	
	Pseudomonas spp	173	
	Proteus spp	49	
	Acinetobacter spp	58	
	Providencia spp	02	
	Enterobacter spp	02	
	Citrobacter spp	08	

Table 3: Year-wise antimicrobial susceptibility pattern of oxidase negative gram negative rods (GNR)

Pathogen	Year and no of isolates (*n*)	Percentages of isolates out of (*n*) showing sensitivity toward									
		Cf	Ctx	Cefo	Ax	Cip	G	Ak	Imp	Mero	PT
Oxidase –ve GNRs	2012 (69)	7.2	15.9	14.5	2.9	18.8	18.8	15.9	60.9	58.0	59.4
	2011 (69)	11.6	26.1	24.6	11.6	29.0	31.9	56.5	72.5	85.5	73.9
	2010 (144)	8.3	18.8	17.4	8.3	18.8	25.0	47.9	85.4	77.1	73.6
	2009 (88)	13.6	23.9	26.1	10.2	26.1	27.3	52.3	87.5	78.4	56.8
	2008 (66)	13.6	22.7	22.7	7.6	33.3	28.8	60.6	90.9	86.4	66.7
	2007 (35)	14.3	25.7	25.7	2.9	31.4	31.4	60.0	97.1	100	97.1
Total	471	10.8	21.4	21.0	7.9	24.6	26.5	48.0	82.0	78.8	69.2

Cf: Cephalexin; Ctx: Ceftriaxone; Cefo: Cefotaxime; Ax: Amoxycillin; Cip: Ciprofloxacin; G: Gentamicin; Ak: Amikacin; Imp: Imipenem; Mero: Meropenem; PT: Piperacillin-Tazobactam

Table 4: Year-wise antimicrobial susceptibility pattern of Pseudomonas spp

Pathogen	Year and no of isolates (*n*)	Percentages of isolates out of (*n*) showing sensitivity toward									
		G	Ak	Cip	Atm	Cfzd	PT	Imp	Mero	Net	Tb
Pseudomonas spp	2012 (22)	54.5	68.2	63.6	63.6	54.5	86.4	100	90.9	86.4	86.4
	2011 (30)	43.3	43.3	46.7	30.0	50.0	86.7	93.3	63.3	53.3	53.3
	2010 (57)	29.8	45.6	38.6	47.4	31.6	63.2	96.5	73.7	56.1	56.1
	2009 (24)	45.8	62.5	37.5	54.2	62.5	45.8	83.3	75.0	45.8	45.8
	2008 (31)	58.1	61.3	54.8	48.4	51.6	58.1	83.9	77.4	67.7	67.7
	2007 (9)	33.3	66.7	22.2	22.2	44.4	33.3	100	100	100	100
Total	173	42.8	54.3	45.1	46.2	46.2	65.3	92.5	75.1	60.1	60.1

G: Gentamicin; Ak: Amikacin; Cip: Ciprofloxacin; Atm: Aztreonam; Cfzd: Ceftazidime; PT: Piperacillin-Tazobactam; Imp: Imipenem; Mero: Meropenem; Net: Netilmicin; Tb: Tobramycin

Table 5: Year-wise antimicrobial susceptibility pattern of MSSA and MRSA

Pathogen	Year and no of isolates (*n*)	Percentages of isolates out of (*n*) showing sensitivity toward											
		Pn	Cf	Cz	Em	Cd	G	Ak	Van	Tei	Lz	Rf	Cm
MSSA	2012 (48)	0	85.4	95.8	79.2	83.3	72.9	91.7	100	100	100	100	100
	2011 (41)	9.8	80.5	92.7	63.4	90.2	78.0	92.7	100	100	100	100	100
	2010 (49)	4.1	91.8	93.9	81.6	91.8	83.7	95.9	100	100	100	100	100
	2009 (27)	0	100	100	88.9	88.9	88.9	92.6	100	100	100	100	100
	2008 (25)	0	92.0	100	64.0	88.0	80.0	92.0	100	100	100	100	100
	2007 (31)	3.2	80.6	93.5	80.6	90.3	74.2	90.3	100	100	100	100	100
Total	221	3.2	87.8	95.5	76.5	88.7	79.2	92.8	100	100	100	100	100
MRSA	2012 (8)	NT	NT	NT	62.5	37.5	25.0	75.0	100	100	100	100	100
	2011 (12)	NT	NT	NT	33.3	50.0	0	41.7	100	100	100	100	100
	2010 (24)	NT	NT	NT	33.3	41.7	16.7	50.0	100	100	100	100	100
	2009 (6)	NT	NT	NT	83.3	83.3	50.0	100	100	100	100	100	100
	2008 (13)	NT	NT	NT	38.5	46.2	7.7	53.8	100	100	100	100	100
	2007 (9)	NT	NT	NT	22.2	22.2	22.2	44.4	100	100	100	100	100
Total	71	NT	NT	NT	40.8	43.7	16.9	56.3	100	100	100	100	100

MSSA: Methicillin sensitive S aureus; MRSA: Methicillin resistant S aureus; Pn: Penicillin; Cf: Cephalexin; Cz: Cefazolin; Em: Erythromycin; Cd: Clindamycin; G: Gentamicin; Ak: Amikacin; Van: Vancomycin; Tei: Teicoplanin; Lz: Linezolid; Rf: Rifampicin; Cm: Chloramphenicol; NT: Not tested

of MRSA (56.3%) toward Amikacin. Majority of the gram negative bacteria showed resistance to most of the first line antibiotics, namely Cefazolin (10.8% sensitivity), Ceftriaxone (21.4% sensitivity), Cefotaxime (21% sensitivity), Amoxicillin (7.9% sensitivity), Ciprofloxacin (24.6% sensitivity) and Gentamicin (26.5% sensitivity). Our study clearly shows that *Acinetobacter spp.* is resistant to most of the first line drugs and the isolates do not even show uniform sensitivity to Imipenem, Meropenem and Piperacillin-Tazobactam. Surprisingly *Pseudomonas spp* showed a gradual increase in sensitivity toward Piperacillin-Tazobactam combination. There was a 33.3% sensitivity toward the drug combination in the year 2007, which rose to 63.2% in 2010 and a further rise to ~87% by 2011 and 2012. It also remained highly sensitive toward Imipenem (92.5% sensitivity) and Meropenem (75.1% sensitivity). A higher infection rate when compared with developed countries may be related to overcrowding in wards, poor socioeconomic status, and lack of hygiene and education. The routine use of Cefazolin (95.5% sensitivity for MSSA) for all orthopaedic patients with open fractures or implant surgery in our hospital is rational as long as the causative organism is expected to be *S. aureus*. Amikacin showed a good amount of activity against gram negative bacteria including *Pseudomonas spp* which have been demonstrated to develop resistance to Ceftazidime, Ciprofloxacin, Aztreonam and Gentamicin as mentioned earlier. When an aminoglycoside is used parenterally, adequate drug concentrations are typically found in bone and synovial fluid. It is often combined with a beta-lactam drug in the treatment of *Staphylococcus aureus* infection.[12] We, therefore, recommend the combined use of Amikacin and Cefazolin as the first drugs of choice for empirical therapy in orthopaedic patients with wound infections.

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