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Study of Bacteria Isolated from Orthopedic Implant Infections and their Antimicrobial Susceptibility Pattern

[1]A.D. Khosravi, [2]F. Ahmadi, [2]S. Salmanzadeh, [3]A. Dashtbozorg and [1]E. Abasi Montazeri
[1]Department of Microbiology,
School of Medicine and Infectious and Tropical Diseases Research Centre,
[2]Infectious Disease Ward, Razi Hospital,
[3]Department of Orthopedic Ward Razi Hospital,
Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran

Abstract: The aim of the present study was, to determine the bacteriology of orthopedic implant infections and susceptibilities of isolated bacteria to the commonly used antimicrobial agents. One hundred and sixty five patients were investigated for early or late postoperative infections of orthopedic bone implants using conventional microbiological procedures. Antimicrobial susceptibility testing were then performed for the isolated bacteria according to the standard guideline. A total of 155 isolates were recovered (152 aerobes and 3 anaerobes). *Staphylococcus aureus*, *Klebsiella ozaenae* and *Pseudomonas aeruginosa* were the most common causative agents. In relation to onset of infection, about 72.9% of patients were with early; 22.6% with delayed and 4.5% with late infections. The correlation between infection onset and total number of isolated bacteria was found to be statistically significant. The majority of isolated bacteria were sensitive to vancomycin, ciprofloxacine and imipenem. In conclusion, present study showed that *S. aureus* was the most common recovered bacterium with high sensitivity to vancomycin as expected. knowledge of the commonly isolated organisms and their antimicrobial susceptibility patterns within a given hospital assists in the selection of appropriate antimicrobial treatment.

Key words: Orthopedic implant, antimicrobial susceptibility, infection, bacteria

INTRODUCTION

Nowadays, implant surgery has become one of the commonest orthopedic operation, because of the success of this procedure in restoring function to the affected joint.

This is the major procedure to alleviate pain and to improve mobility in people with damaged joints (Goel, 2006). Less than 10% of prosthesis recipients develop implant-associated complications during their lifetime, predominantly as aseptic failure. Infections associated with prosthetic joints occur less frequently than aseptic failures, but represent the most devastating complication (Trampuz and Zimmerli, 2005). Bacterial infection has been a significant part of this complication implicated in 22% of revision operations in a recent study (Brause, 2005).

Orthopedic implant infections are significant because of their morbidity and a tendency to serious relapses (Gómez *et al*., 2003). It can also be an economic disaster for hospitals that treat large numbers of these patients. The most important factor in both the clinical and economic area is to prevent the infection from occurring at all. However, once deep infection is established, rapid, aggressive and

Corresponding Author: Azar Dokht Khosravi, Department of Microbiology,
School of Medicine and Infectious and Tropical Diseases Research Centre,
Ahwaz Jondishapour University of Medical Sciences, Ahwaz, Iran
Tel: +98 611 3330074 Fax: +98 611 3332036

definitive treatment must be rendered to the patient. In addition to protracted hospitalization, patients risk complications associated with additional surgery and antimicrobial treatment, as well the possibility of renewed disability (Widmer, 2001).

A major risk factor for local infection is the extent of the soft tissue and periosteal damage associated with the fracture. Devascularised bone or other necrotic tissue is an ideal matrix for bacterial growth. Damage to the periosteal blood supply and lack of perfusion of the soft tissues will not only interfere with fracture healing but also prevent the humoral and immunological host defense mechanisms from reaching the traumatized area and fighting the spread and multiplication of inoculated microorganisms at the bone-implant interface (Arens *et al*., 1999).

In regards to patient factors, several conditions has been recognized to significantly increase the risk of postoperative infection as: Rheumatoid arthritis, diabetes mellitus, sickle-cell anemia, psoriasis, renal failure with hemodialysis, immunosuppression due to prior renal or liver transplant, malnourishment, obesity, concurrent urinary tract infection (bladder retention in post-operation), malignancy and postoperative surgical infection (Berbari *et al*., 1998).

Staphylococcus epidermidis and *Staphylococcus aureus* are the most common offending organisms, whereas *Streptococcus viridans*, *Escherichia coli*, *Enterococcus faecalis* and group B streptococci are less frequently encountered. About one-third of these infections develop within 3 months, another third develop within 1 year and the remainder develop more than 1 year after surgery (Goel, 2006).

Removal and replacement of the prosthesis are usually required to eradicate the infection with attendant patient trauma and increased cost. Antibiotic treatment to reduce the risk of recurrent infection includes the use of antibiotic-impregnated bone cement for prosthesis fixation at revision surgery and the intravenous administration of antibiotics during revision surgery (Tunney *et al*., 1998).

The aim of the present study was to determine the bacteria isolated from orthopedic implant infections and susceptibilities of these bacteria to the commonly used antimicrobial agents.

MATERIALS AND METHODS

In this cross sectional descriptive study undertaken in Ahwaz University of Medical Sciences teaching hospitals, Iran, 165 patients with orthopedic implant infection who needed hospitalization and surgical debridement, from June 2007 to April 2008 were investigated. Patients with the history of more than one surgery for the same implant, using antibiotic 48 h prior to sampling and immunodeficiency, were excluded from the study. According to type of implantation, 136 patients (82.4%) had prosthesis or internal fixation and the rest 29 patients (17.6%) had external fixation.

Despite that the sampling was a part of patients' diagnosis protocol, informed consent was taken in the presence of the research assistants. Besides, permission was obtained from human ethics committee at the university during the approval of the proposal.

Samples for bacteriological examination were obtained from secretions adjacent to the infected implant and tissue by an sterile cotton swab in the operating room. Samples were collected in Thioglycollate broth medium (Merck, Germany) and immediately transferred to microbiology department and was incubated at 37°C for 24 h in order to enrich bacterial cells. Subcultures were made on duplicate Blood agar plates, one aerobic and one anaerobic and onto Chocolate agar plate, MacConkey's agar and Mannitol salt agar plates (Merck, Germany). The aerobic blood agar and chocolate agar plates were incubated at 37°C in presence of 10% CO_2 for 48 h and the anaerobic plate, was placed in an anaerobic chamber at 37°C for 5 days. Organisms were then identified using routine laboratory methods including conventional biochemical tests (Forbes *et al*., 2007).

The bacteria found in each sample were identified and tested for antimicrobial susceptibility by means of agar disc diffusion method of Kirby Bauer according to the guidelines of Clinical and Laboratory Standards Institute (2002). The following antimicrobial agents were used: gentamicin,

vancomycin, cefazoline, ceftriaxone, ceftazidime, imipenem, piperacillin, clindamycin, ciprofloxacine, cloxacillin and cotrimoxazole. All the antibiotics were purchased from Patan Teb, Tehran, Iran.

All criteria were analyzed by SPSS software using Chi-square test and Fischer's exact test.

RESULTS

From the total number of patients investigated in present study, 68.5% were males and 31.5% were females and their age ranged from 16 to 80 years with a mean age of 39.3 years. Based on the results from culture, 155 patients (93.9%) had a positive culture, while in 10 patients the culture was negative (6.1%). Among the positive cultures, less than 2% were mixed bacterial culture of two organisms and one culture was comprised of more than two organisms. The positive bacterial cultures were frequently detected in patients with femural (52.1%) and tibial (37.6%) fractures. Infection in other fractures was less frequent.

According to the patients' records, the noted risk factors were smoking 33.3%, diabetes 20.6% and drug addiction 1.8%.

Bacteriological cultures revealed that, the most prevalent isolated bacteria were Gram-negative bacilli including *Pseudomonas aeruginosa* and *Klebsiella ozaenae* and among Gram-positive recovered cocci, *S. aureus* was the most common. Table 1 shows the incidence of isolated bacteria. Giulieri *et al*. (2004) have earlier been classified the onset of infection after implantation into three categories as: early (less than 3 months), delayed (between 3 to 24 months) and late (after 24 months). Based on this classification, the onset of infections appeared in our patients in this study were as: 72.9% early, 22.6% delayed and 4.5% late. The prevalence of isolated bacteria in relation to onset of infection symptoms are shown in Table 2.

Table 1: Prevalence of isolated aerobic and anaerobic bacteria from orthopedic bone implant infection

Bacteria	No. (%)
S. aureus	34 (21.94)
Klebsiella ozaenae	26 (16.77)
P. aeruginosa	24 (15.50)
E. coli	23 (14.83)
S. epidermidis	14 (9.05)
Enterobacter cloacae	11 (7.10)
P. mirabilis	9 (5.80)
Acenitobacter baumannii	7 (4.51)
Streptococcus viridans	3 (1.93)
Bacteroides	3 (1.93)
Entrococcus faecalis	1 (0.64)
Total	155 (100.00)

Table 2: Prevalence of isolated bacteria from orthopedic bone implants in relation to onset of infection

Bacteria	Early No.*	Delayed No.**	Late No.***	Total
S. aureus	16	16	2	34
Klebsiella ozaenae	25	0	1	26
P. aeruginosa	21	3	0	24
E. coli	18	5	0	23
S. epidermidis	9	4	1	14
Enterobacter cloacae	9	2	0	11
P. mirabilis	8	1	0	9
Acenitobacter baumannii	7	0	0	7
Streptococcus viridans	0	3	0	3
Bacteroides	0	0	3	3
Entrococcus faecalis	0	1	0	1
p-value	<0.001	0.245	0.746	

*Less than 3 months; **Between 3 to 24 months; ***After 24 months

Table 3: Susceptibility results of various bacteria isolated from orthopedic implants to commonly used antibiotics

	Antibiotic sensitivity (%)										
Organisms	CFZ	CRO	CAZ	IPM	PRL	VCM	CLM	CIP	GM	CLX	SXT
S. aureus	8.8	8.8	0.0	38.2	14.7	99.4	47.1	41.2	2.9	5.9	5.9
Klebsiella ozaenae	3.8	11.5	26.9	94.6	0.0	0.0	0.0	46.2	0.0	0.0	16.0
P. aeruginosa	0.0	0.0	45.8	100.0	58.3	0.0	0.0	79.2	29.2	0.0	12.0
E. coli	0.0	21.7	30.4	98.3	26.1	0.0	0.0	69.6	21.7	0.0	21.7
S. epidermidis	0.0	14.3	0.0	71.4	21.4	98.6	35.7	50.0	35.7	0.0	50.0
Enterobacter cloacae	0.0	18.2	27.3	100.0	0.0	0.0	0.0	72.7	27.3	0.0	0.0
P. mirabilis	0.0	77.8	44.4	100.0	66.7	0.0	0.0	88.9	77.8	0.0	75.0
*Acenitobacter	0.0	0.0	57.1	100.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Strep. viridans	0.0	0.0	0.0	100.0	100.0	100.0	100.0	100.0	0.0	0.0	0.0
Bacteroides	0.0	0.0	0.0	33.3	66.7	33.3	100.0	66.7	0.0	0.0	0.0
**Entrococcus	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0

**Acenitobacter baumannii*, ** *Enterococcus faecalis*. CFZ: Cefazoline, CRO: Ceftriaxone, CAZ: Ceftazidime, IPM: Imipenem, PRL: Piperacillin, VCM: Vancomycin, CLM: Clindamycin, CIP: Ciprofloxacine, GM: Gentamicin, CLX: Cloxacillin, SXT: Cotrimoxazole

Statistical analysis revealed that there was no significant correlation between onset of infection or type of bacteria with patients' gender ($p = 0.22$) or age ($p = 0.24$). This correlation was found to be highly significant between onset of infection (early type) and number of positive cultures and type of recovered bacteria ($p<0.001$). Similarly the statistical significant correlation was found between patients' age group and site of infection. The infection of femur fractures was more common in patients in age group of 41-60 ($p<0.001$).

While the majority of bacterial isolates showed resistance to many tested antimicrobials, they overall were more sensitive to imipenem, vancomycin and ciprofloxacine, with *Streptoccus viridans* showed full sensitivity to these antibiotics plus clindamycin (Table 3).

DISCUSSION

Implant-related infection is an unresolved problem in the development of orthopedics. Infections occur even though orthopedists perform thoroughly clean procedures during surgery and patients are strictly managed before and after surgery (Nishimura *et al*., 2006). Phillips *et al.* (2003) reported that among patients who had primary total hip replacement, 0.2% of 58521 had a deep infection during the first 26 postoperative weeks.

According to present results, positive culture were seen on majority of the studied patients (93.9%), while in the study of Gomez *et al.* (2003) reported positive cultures was 60%. The finding of Zimmeli *et al.* (2004) was close to us, however the reported value was 89%.

In total 748 implant surgeries were performed during the study. While the radius was the most frequently fractured bone, only 8.9% of positive cultures were belonged to these bone implants. On the other hand, fractured femur which had accounted for 27.9% of total implant surgeries, was the most common site for bacterial infection (52.1%).

Despite that the patients with open fractures tended to have a higher risk of bone infection due to easy access of bacteria into the bone, than those with closed fractures, the incidence of infection in open and external fractures were as only 17.6%, while 82.4% of implant infections were occurred in closed and internal fractures in present study. Additionally, an association with severe soft tissue damage in these patients puts them at a higher risk of infection. These types of infections were mainly occurred in patients with diabetes and with less frequency in smokers and drug addicts and significant correlation was found between internal fractures and infection occurrence ($p<0.001$).

In present study the most prevalent infection was early onset (72.9%). In previous studies, the onset of early infection was reported as one third of total infections and 29%, respectively (Gómez *et al*., 2003; Giulieri *et al*., 2004). The high prevalence of early infection in this study, may be related to inadequate disinfection procedure to eliminate microorganisms from the environment,

contamination of surgical instruments and /or contaminated implants. Additionally, trauma and fracture fixation using metallic implants may produce structural and functional damage to the local host tissue causing devascularization, malperfusion, disturbance of endothelial permeability, hypoxia, acidosis, haematoma, edema and increased intra-compartmental pressure. This may result in an impaired humoral and cellular immune competence (Valenziano *et al*., 2002). On a local level it may decrease resistance to the pathogenic microbiological load with subsequent manifestation of infection in the traumatized tissue and put the patients at a higher risk of early infection.

Based on bacteriology findings, The prevalence of isolated bacteria were as: aerobic Gram-Positives 33.5%, aerobic Gram-negatives 64.5% and anaerobes 1.9%. This was in controversy with Gomez *et al*. (2003) study with the different incidence rate for Gram-positive and Gram-negative bacterial isolates as 60.6 and 33.3%, respectively, probably due to different nosocomial pathogens present in our operating rooms. Besides the rate of anaerobic isolates in their study was higher, since, the majority of implant infections was late onset (67%).

Staphylococcus aureus was frequently found in present study, followed by *Klebsiella ozaenae* and *P. aeruginosa*, which we assumed that these were the main nosocomial pathogens in our operating room. Present findings were in agreement with the extensive study of Arciola *et al*. (2005) which reported staphylococci as the most prevalent organism and study of Mousa (2001) that reported *P. aeruginosa* as the significant isolated organism. However, this organism was the third most prevalent bacterium in present study. The anaerobic bacteria isolated in present study was not high and the rate was 1.9% of total isolated organisms. Interestingly all these were isolated from patients with late onset of the implant infection reflecting that anaerobic microorganisms appear to play a significant role in the pathogenesis of late-onset postoperative infection in this study, especially where there is an extra medullary internal fixation device.

Antimicrobial susceptibility test revealed the high rate of antimicrobial resistance in this study suggesting a horizontal spreading of resistance among the isolates. It is found that most of the organisms were susceptible to imipenem and ciprofloxacine. Moreover, the staphylococci which was the major isolate, showed high sensitivity to vancomycin. Present findings is similar to a earlier study reported vancomycin and ciprofloxacine as the most effective antibiotics against isolated bacteria (Tunney *et al*., 1998). Since, most of the common antibiotics such as clindamycin, cephalosporins and gentamycin which are normally used for treatment of such infections were ineffective against bacteria, so, we suggest a combination of vancomycin with imipenem or ciprofloxacine for treatment coverage of the majority of isolated bacteria in present study.

In conclusion, knowledge of the commonly isolated organisms and their antimicrobial susceptibility patterns within a given hospital assists in the selection of appropriate antimicrobial treatment.

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