www.jmscr.igmpublication.org
Impact Factor 5.84
Index Copernicus Value: 83.27
ISSN (e)-2347-176x ISSN (p) 2455-0450
crossref DOI: https://dx.doi.org/10.18535/jmscr/v5i4.64

IGM Publication
Journal Of Medical Science And Clinical Research
An Official Publication Of IGM Publication

A Study on Bacteriological Spectrum of Post-Operative Orthopaedic Implant Infections and their Antibiotic Sensitivity Pattern in a Tertiary Care Hospital

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Abstract
Background: *Post operative infections in orthopedic implants is a major problem in orthopedic patients nowadays which can lead to implant failure and in severe cases can even lead to amputation and mortality.These are mainly associated with Open reduction and internal fixation (ORIF) of fractures with implants and prosthesis which has become the first line in the management of fractures in most traumacentres in recent times. This is also associated with high morbidity and cost for patient during his hospital stay.*
Objectives: *The objective of this paper is to isolate and identify the bacteriological isolates responsible and their antimicrobial sensitivity from post-operative orthopedic implant infections.*
Materials and Methods: *This was a prospective study carried out at a tertiary care hospital in India over a period of six months. The study was conducted on 50 cases of infected implants from orthopaedic wards, admitted in DR.B.R Ambedkar Medical College, Bangalore from 1st July to 1st December 2016.Pus samples were collected using two sterile swabs .One is used for Grams stain and other for inoculation on Mac conkey and blood agar. Susceptibility testing was performed by Kirby-Bauer disk diffusion technique.*
Results: *Out of the 50 samples processed, 40 (80%) of specimens showed culture positivity. Staphylococcus aureus 14(35%) was the predominant isolate followed by Escherichia coli 10(25%),Klebsiellaspp 8(20%), Pseudomonasspp 5(12.5%),Acinetobacterspp2(5%) and Proteus spp1 (2.5%). All Gram-positive cocci were susceptible to vancomycin and linezolid.*
Gram negative bacilli were resistant to ceftriaxone (84.6%), ciprofloxacin(69.2%), cotrimoxazole (69.2%)and sensitive to carbapenems and piperaciillintazobactam. ESBL production is seen in 11(61%) cases of Gram negative bacteria.
Pseudomonas isolates were susceptible to piperacillin-tazobactam, and meropenem.
Conclusion: *Orthopedic implant post-operative infections are a major concern in the present scenario. There is an increase incidence of multidrug resistance among the pathogens isolated from these isolates. Adequate preventive measures should be taken to prevent these antibiotic resistance amongst organisms. In this study Gram Positive Organisms has emerged as major threat for orthopedic implants.*
Key Words: *Bacterial isolates; Antibiogram; Orthop edic infection; Wounds; ESBL.*

Introduction

Infection is a major concern in orthopedic implants leading to implant failure. It is very difficult to treat orthopedic implant infections which may lead to implant failure. Sources of infection include environment of operating room, surgical equipments, clothing worn by medical and paramedical staff. Implant related infections can be due to biofilm formation also which is very common nowadays. In most of the cases removal of the infected prostheses is the ideal solution to cure these infections.

Orthopedic infections are one of the most common which can occur in approximately 1% of all orthopedic operations.[1] The most common orthopedic infections are surgical site infections (SSI) and implant infections in open or closed wounds. [2,3] Wound is a breach in the skin leading to exposure of subcutaneous tissue caused by trauma, surgeries, burns, diabetic ulcers, etc. It provides a moist, warm and nutrient environment that is conductive to microbial colonization and proliferation that leads to serious bacterial infections and death. Wound infections are one of the most common hospital-acquired infections morbidity and account for70-80% mortality.[4]

Surgical site infection (SSI) as defined by US Centers for Diseases Control (CDC) in 1992 is an infection occurring within 30 or 90 days after a surgical operation (or within 1year if an implant is left in place after procedure) and affecting either incision or deep tissues at the operation site. These infections may be superficial or deep incisional infection or infections involving organ or body space. [5]

Open or compound fractures are fractures that communicate with the outside environment through skin wounds. [6] The main causes of open fracture include road traffic accidents, fall from height, assaults, machine injury and others. Anglen JO et al reported 3-4% of all fractures are open fractures and the development of infection is favored by devitalization of bone and soft-tissue. Use of implants and prosthesis during the orthopedic surgeries can pose greater risk of microbial contamination and infection. [7]

Numerous studies have documented that gram-positive organisms are the most common bacteria causing infections associated with joint arthroplasty, with Staphylococcus aureusand Staphylococcus epidermidis causing the majority of the infections. [8,9-11] Enterococcus, Streptococcus, and gram-negative organisms such as Escherichia coli, Pseudomonas species, and Klebsiella species are less common but have been frequently reported [12]. These microorganisms can all be part of normalskin flora; hence, direct inoculation at the time of the operation as well as airborne contamination are the most likely causes of these infections. Although Staphylococcus epidermidis is generally not considered pathogenic, infections surrounding a joint replacement prosthesis may be more difficult to treat because of the bacterial biofilms typically produced by Staphylococcus aureus and Staphylococcus epidermidis around orthopaedicimplants [13,14]. This glycocalyx layer, which is formed on the surface of the orthopaedic devices, creates a complex environment for the bacteria. Numerous factors, including restricted penetration of antimicrobials into the biofilm, decreased bacterial growth rates, and expression of biofilm-specific resistance genes, all contribute to bacterial and biofilm resistance [15].

In addition to the irrational use of broad spectrum antibiotics, the changing pattern of microbial etiology and increasing challenge for both the patient and clinician.

In recent years the organisms from these infected cases are showing increased resistance to commonly used first line drugs and multi drug resistance. Methicillin resistance has become most common and also ESBL producers.

So the present study was conducted to delineate the occurrence and sensitivity pattern of such infections for a better management, thereby reducing both mortality and cost issues.

Materials and Methods

Study center

This was a prospective study carried out at a tertiary care hospital in India over a period of six months. (from 1st July to 1st December 2016).

Patient's selection

All the patients who had close fractures of long bones treated by ORIF with purulent discharge from incision or drain within a week after surgery or after few weeks after discharge from hospital of all age groups and both sexes were included into the study.

Patients with use of antibiotics after diagnosis of infection were excluded. Intra-operatively, cefuroxime (Zinacef) or Ceftriaxone (Rocephin) were used for perioperative antibiotic prophylaxis.

Processing of specimens

Swabs from open fractures, bed sores and wounds clinically suspected to be infected were collected with all aseptic precautions to avoid contamination and were immediately transported to the Microbiology laboratory. The pathogens were identified by standard laboratory procedures including gram's staining, motility, colony characters and biochemical reactions. for culture the specimens were inoculated into mac conkey and blood agar. Antibiotic susceptibility testing was done by Kirby-Bauer disc diffusion method as per CLSI guidelines[16]. Following antimicrobials were used

Table 1-The concentration of the antibiotics employed were as per CLSI guidelines .[16]

The drugs used for Gram positive organisms Were:	The drugs used for Gram negative organismswere:-
Azithromycin (AZM) 15 µg	Ampicillin (AMP) 10 µg
Clindamycin (CD) 2 µg	Gentamicin (GEN) 10 µg
Cefoxitin (CN) 30 µg	Tobramycin (TOB) 10 µg
Penicillin (P) 10 units	Amoxycillin - Clavulanic acid (AMC) 20 µg + 10 µg
Co-trimoxazole (COT) 25 µg(1.25/23.75 µg)	Ampicillin – Sulbactam (AS) 10 µg + 10 µg
Linezolid (LZ) 30 µg	Cefoxitin (CN) 30 µg
Vancomycin (VA) 30 µg	Cefotaxime (CTX) 30 µg
Tetracycline (TE) 30 µg	Cefepime (CPM) 30 µg
Levofloxacin (LE) 5 µg	Ceftazidime (CAZ) 30 µg
Gentamicin (GEN) 10 µg	Levofloxacin (LE) 5 µg
Gentamicin (HLG) 120 µg	Co-trimoxazole (COT) 25 µg
Erythromycin(E) 15µg	Piperacillin (PC) 100 µg
Teicoplanin(TEI)30 µg	Piperacillin – Tazobactam (PIT) 100/10 µg
Amoxyclav(AMC) 50/10 µg	Imipenem (IPM) 10 µg
	Meropenem (MRP) 10 µg
	Aztreonam (AT) 30 µg.
	Norfloxacin(NX)-10 µg
	Nitrofurantoin(NIT)-300 µg

For Pseudomonas species

Ceftazidime (30mg), gentamicin (10mg), amikacin (30mg), piperacillin/tazobactam (100mg/10mg), cefepime (30mg), cefoperazone/ sulbactam (75mg/30mg), aztreonam (30mg), ofloxacin (5mg), imipenem (10mg),ceftriaxone (30mg), netilmicin (30mg), ceftizoxime (30mg). All the antibiotic discs used were obtained from Hi-Media Laboratories Pvt. Ltd.

Detection of MRSA[17]

Inoculum was prepared by emulsifying 2-3 identical colonies in the broth. Inoculum turbidity was adjusted to 0.5 McFarland turbidity tube. A lawn culture was made on the surface of the MHA agar plate using sterile cotton swab and 30 µg Cefoxitin antibiotic disc was applied. The antimicrobial discs were obtained from Hi Media Laboratories Private Limited, Mumbai. The plates were incubated for 18-24 hours at 37 0 C. After 24 hours reading was taken and zone of inhibition was read and reported. The diameter of each zone (including the diameter of the disc) of inhibition was measured and recorded in millimeters and the result was then compared with the zone size interpretative chart.

If Zone if inhibition of cefoxitin is > 22 mm it is sensitive. If < 21 mm then reported as Methicillin Resistant. The concentration of the antibiotics employed were as per CLSI guidelines

Detection of ESBL[18]

Phenotypic Confirmatory test was followed using Cefotaxime 30 µg Cefotaxime-clavulanate 30/10 µg and Ceftazidime 30 µg Ceftazidime-clavulanate 30/10 µg. Standard Disk diffusion procedure followed and the disks were placed on MHA on which a lawn culture of the test organism was done. The plates were incubated for 18-24 hours at 37 0 C. After 24 hours reading was taken and zone of inhibition was read .A≥ 5-mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanate vs the zone diameter of the agent when tested alone=ESBL .

Results

Out of the 50 samples, 40(80%) culture were positive and 10(20%) culture were negative.

Out of 40 culture positive cases, 31 (77.5%) were males and 9(22.5%) cases were females. Out of 40 culture positives, 14 (35%)were Gram positive cocci and 26(65%) were Gram negative bacilli.

Staphylococcus aureus (35%) was the most common isolate followed by E coli(25%), Klebsiellaspp(20%) , Pseudomonasspp (12.5%), Acinetobacterspp(5%) and Proteusspp (2.5%).

Antibiogram of gram positive cocci showed highest resistance to Penicillin[100%], Amox-clav [71%], Cotrimoxazole [71%], Levofloxacin [57%] and Gentamicin[57%].Vancomycin, teicoplanin and linezolid did not show any resistance. MRSA was seen in (71.42%)% cases of Staphylococcus aureus.

About 100% of E.coli was sensitive to imipenem and 70 % sensitive to nitrofurantoin. However it was found resistant to ceftriaxone (90%), Ciprofloxacin (80%) and cotrimoxazole (80%). Among aminoglycoside, amikacin and gentamicin showed good sensitivity (70 %). Klebsiellaspp was equally sensitive (75%) to nitrofurantoin and amikacin and gentamicin (87%). Proteusspp was sensitive to most of the antibiotics. However, number of isolates was very small to draw a definitive conclusion. Proteus mirabilis was also sensitive to most of antibiotics with 100% sensitive to amikacin, cotrimoxazole and piperacillin-tazobactam.

Among E coli, 6(60%) cases were ESBL and Klebsiellaspp 5(62.5%) were ESBL.

Acinetobacter baumanii isolates were found to be highly sensitive (100%) imipenem. It was highly resistant to, ciprofloxacin (50%) and cotrimoxazole (50%). Pseudomonas aeruginosa isolates were sensitive to most of the antibiotics.

Table 2: Distribution of culture positive and culture negative samples

	cases	percentage
Growth	40	80%
No growth	10	20%
Total	50	100%

Table 3: Sex distribution

	Male	Female
Total cases(40)	31(77.5%)	09(22.5%)

Table 4: Distribution of single bacterial isolates

Bacteria	Isolate No. (%)
Staphylococcus aureus	14(35%)
Escherichia coli	10(25%)
Klebsiella species	08(20%)
Pseudomonas species	05(12.5%)
Acinetobacter spp	02(5%)
Proteus spp	01(2.5%)
Total	40(80%)

Table 5: Antibiotic resistance pattern of bacterial isolates

Isolate	CTX	LEV	GEN	NIT	NX	AK	CPM	CAZ	IMP	COT	CIP	PTZ
E. Coli	09	05	03	03	05	03	02	02	0	08	08	0
Klebsiellaspp	06	04	02	02	04	01	01	01	0	05	06	0
Pseudomonas spp	04	03	02	01	01	01	03	02	0	03	03	0
Acinetobacter	02	02	02	01	02	01	02	02	0	01	01	0
Proteus spp	01	01	0	0	0	0	01	01	0	01	0	01

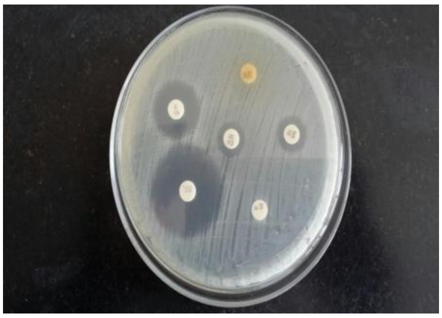
Table 6: Antibiotic resistance pattern of gram positive isolates

Organism	P	AMC	COT	CX	E	VA	TEI	LZ	LE	GEN	NIT	NX
S.aureus(14)	14	10	10	8	10	0	0	0	08	08	09	10

Table 7: Distribution of ESBL isolates:

Isolates	ESBL	NON-ESBL
E.coli (10)	6(60%)	4(40%)
Klebsiella (8)	5(62.5%)	3(37.5%)
	11(61.1%)	7(38.9%)

Fig 1 Disk diffusion test for MRSA detection



Discussion

In our study, out of 50 samples, 40 (80%) were culture positive.

This is similar to study conducted by Devi et al [19], in which out of 100 samples, 68% samples yielded growth. Among them, predominant organisms were Gram-negative bacilli with Pseudomonas (18 isolates) being most common organism with the highest sensitivity to piperacillin + tazobactam, imipenem and amikacin. Among the Gram-positive organisms isolated, S. aureus (17 isolates)

was the most common organism with maximum sensitivity to vancomycin and linezolid. Abraham Y et al (20) study which showed 41% positivity, whereas Gomez et al (21) and Zimmeli et al (22) reported positive cultures in 60% and 89% respectively.

Staphylococcus aureus was the most commonly isolated micro-organism in this study accounting for 35%. This was similar to a study conducted by Sonawane et al [23] where staphylococcus was the dominant organism (29.26%). This was similar to study conducted by Goel et al [24] 2013(32.8%).

But there are few studies where gram negative organisms were isolated the most. In a study conducted by Suneet Tandon et al [25%], Klebsiella (39.53%) was most isolated species. In our study maximum resistance is shown to penicillin among gram positive cocci which is similar to study conducted by Sonawane et al in 2010 and Jain et al 2014

In our study (71.42%) were MRSA, which does not correlate with other studies. Bergqvist et al (26) and Dan et al (27) found that 29.8% of hospitalized patients and 26.6% of hospital staff respectively are carriers. 12.5% (11/88) of our isolates are Methicillin Resistant Staphylococcus aureus. ESBL production is (61.1%) which corelates with Sonawane [23] et al 2010(71.72%).

The gram-negative aerobic rods like E.Coli, Pseudomonas, Proteus and Klebsiella were found to be sensitive to amikacin while essentially resistant to the cephalosporin tested. this was similar to study conducted by Satya Chandrika et al. [28] From our results, we observed that amoxicillin/clavulanic acid, ceftriaxone and ceftazidime cannot be recommended for use as an empirical therapy in SSI and open fracture infections because these drugs were inactive against most strains. Based on the antimicrobial susceptibility data, we suggest that piperacillin/ tazobactum and imipenem are the most effective agents against most of gram negative bacteria and vancomycin, teicoplanin and linezolid are the most effective agents against gram positive organisms.

Conclusion

As there is high antibiotic resistance observed in our study, it is necessary for routine microbial analysis of samples and their antibiogram. Multidisciplinary collaboration with orthopedic surgeons, infectious disease specialist and clinical microbiologist is needed to reduce the incidence of orthopedic infections. There is a need for formulation of antibiotic policy and formulary restriction.

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