ORIGINAL RESEARCH

Characterization of bacteria isolated from orthopaedic implant associated infections and their antimicrobial pattern

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ABSTRACT
Hundreds of thousands of patients undergo joint replacement surgeries each year,worldwide and millions of people have anindwelling prosthetic articulation. Prosthetic jointinfections (PJIs) are devastating complications which follow each surgery. The isolates were identified by colony morphology, Gram's stain and biochemicalreactions and antibiotic susceptibility tests performed by CLSIrecommended by Kirby-Bauerdisc diffusion method.
Out of 77 clinically suspected cases of Orthopaedic infected implant, 70 cases showed positiveculture and 7 cases were culture negative. Among 70 isolates, most common was*Staphylococcus aureus* 32 (45.71%), followed by *Proteus mirabilis* 8(11.42%), *CONS* 07(10%), *E.coli*and *Pseudomonas aeruginosa* 06 each (8.57%),(5.43%), *Klebsiella*sppand *Citrobacter*spp 04 each (5.71%) and *Streptococcus* spp 03 (4.28%)*Staphylococcus aureus* was the predominant pathogen isolated from the Orthopaedicimplant infections from our hospital.
Key words: Orthopaedic infected implants, antibiotic susceptibility, Methicillin-Resistant*Staphylococcus aureus*(MRSA)

Introduction

A prosthetic replacement and an implant surgery is common place in Orthopaedicoperations for successfully alleviating the pain and improving the mobility in damaged joints[1]. Inmodern era implant surgery has become one of the commonest Orthopaedic operation[2].Thedevelopment of prosthetic joints has been one of the biomedical succcess stories of the century,with a major health-economic and quality-of-life-benefits[3].

Although prosthetic joint implantation has become an important medical procedure thatimproves quality of life for many patients, the majority of failures that lead to severe consequences remain unsolved[4].

Infection continues to plague all medical disciplines that rely on implantation of a foreign

Object[5].In Orthopedics, the surgical site infection after implant surgery is a disaster both for the patient and surgeon. This may lead to prolonged hospital stay, repeated debridements, prolong rehabilitation, morbidity and mortality[6].Prostheticjoint infection (PJI) is an uncommon complication (1-2%) of joint replacement surgery, and isassociated with high morbidity and medical cost [7].

Main risk factors for occurrence of infection are advanced age, diabetes mellitus,

smoking, malnutrition, obesity,immune repairment, rheumatoid arthritis, infection in other partof body, and anemia[8].Extrinsic and intrinsic risk factors associated with Orthopedic infectioninclude the patient's clinical conditions, prolonged preoperative hospitalization time, surgerylength, skin preparation and team's hand degerming technique, environmental conditions of operating room, number of people inside the room, surgeon's technique and skills, use ofimplants, among others[9]. SSIs are an important cause of increased hospital stay, and they directlyaffect the morbidity and risk of mortality of surgical patients, particularly older patients[10].

An early SSI present within 30 days of surgical procedure, where as an infection isdescribed as intermediate if it occurs between one and three months and late if it develops morethan three month after surgery. Early infections are mainly caused by

highly virulentmicroorganisms eg. *Staphylococcus aureus*and gram negative bacilli, while delayed and late SSI are caused by low virulence microorganism like coagulase-negative staphylococci [8]. Productionof slime is characteristic of many strainsof *S.epidermidis* and *S.aureus*. Transmission electronmicroscopic examination of antibody-stabilized biofilm preparation revealed that theexopolymeric matrix appears as fine fibres providing relatively thick, hydrated coatings aroundthe cells. The ability to form a biofilm on the surface of a prosthetic device is probably asignificant determinant of virulence for these Staphylococci[11].

Staphylococcus epidermidis(31%) and *Staphylococcus aureus* (20%) are the mostcommon offending organisms, whereas *Streptococcus viridians* (11%), *Escherichia coli*(11%),*Enterococcus faecalis (8%),* and group B *Streptococci*(5%) are less frequently encountered.

Infection of bone have been known for a long time. Robson (1979) described infectionas the result of an imbalance between an overwhelming number of virulent bacteria and localdefence mechanisms. In the classical experiment, of Elek and Conen (1957) the implantation of foreign bodies was shown to increase the susceptibility to infection.

Orthopaedic implant site infection is one of the major constituent of surgical siteinfection associated with high morbidity and mortality. Due to the use of implants for openreduction and internal fixation, which are foreign to the body, Orthopaedic trauma surgery is atgrave risk of microbiological contamination and infection.

Even aggressive medicosurgical treatments are not always able to guarantee permanenteradication of the infectious process, particularly when these infections occur in patients withforeign Orthopaedic material.

With the discovery of Penicillin in 1940 the incidence of bacterial infection decreasedworldwide until *Staphylococcus aureus* (S.aureus) began producing an enzyme, beta-lactamase,that destroys Penicillin. Increasing resistance to Penicillin has led to the development of semi-synthetic groups ofPenicillin such as Methicillin. However, in 1961 the first strain ofMethicillin resistant *S.aureus* (MRSA) was isolated. Since then MRSA has been foundworldwide especially in hospitals and nursing homes[12].

Methodology

Approval was obtained from the institutional ethical committee before the commencement ofthe study.Informed consent was obtained from the study population.All patients satisfying theinclusion criteria were documented. Patients were interviewed by structured questionnaire.

Study population

Patients admitted with orthopaedic implant infection in orthopaedicpost operative and septicward.

Case definition

Diagnosis of orthopaedic implant infection is based on clinical data (pain,swelling and warmthofthe joint, discharge and fever), together with one or more of the parameters mentioned below:elevated ESR, elevated C-reactive protein and leukocytosis over 12,000 or WBC less than 4000cells.

Inclusion criteria

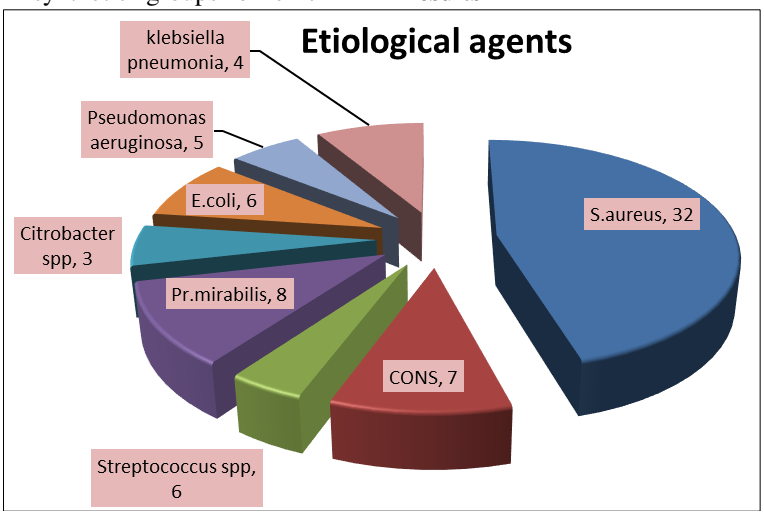
Patients with infected Orthopaedic implants in Post-Operative and septic Orthopaedic wards.

Exclusion criteria: Isolation of polymicrobial flora.

Data collection

Data collection included name, age, address, date of admission, diagnosis at admission, physicalexamination finding. Date of surgery, duration of hospital stay, nutritional status, underlyingillness (diabetes mellitus, uremia, chronic arthritis and concurrent urinary tract infection), type ofimplant, duration of procedures, smoking and alcoholism were also recorded.

Results



Graph 1: Etiological agents of Orthopaedic implant infections

Out of 77 clinically suspected cases of Orthopaedic infected implant, 70 cases showed positiveculture and 7 cases were culture negative. Among 70 isolates, most common was*Staphylococcus aureus* 32 (45.71%), followed by *Proteus mirabilis* 8 (11.42%), CONS 7 (10.00%),*E.coli*and *Klebsiella*spp 6 each,(8.57%), *Pseudomonas aeruginosa* and *Citrobacter*spp 4 each (5.71%) and*Streptococcus* spp 03 (4.28%),

Table 1: Antimicrobial sensitivity patterns of GPC

Antibiotics	***Staphylococcus aureus* n=32**		**CONS n=7**		***Streptococcus*spp n=03**	
Amikacin	26	81.25%	4	57.14%	03	100%
Ciprofloxacin	20	56.25%	5	71.42%	03	100%
Cotrimoxazole	03	9.37%	3	42.85%	01	33.33%
Clindamycin	4	12.5%	4	57.14%	03	100%
Erythromycin	5	15.62%	3	42.85%	03	100%
Vancomycin	32	100%	7	100%	03	100%
Cefotaxime	26	81.25%	3	42.85%	01	33.33%
Ceftazidime	25	78.12%	3	42.85%	01	33.33%
Linezolid	32	100%	7	100%	03	100%

In this study, among gram positive isolates *Staphylococcus aureus* and CONS were commonlyisolated and were found to be 100% sensitive to Vancomycin and Linezolid.

Among *S.aureus* isolates, 81.25% were sensitive to Amikacin, Cefotaxime, 78.12% toCeftazidime, 56.25% to Ciprofloxacin, 15.62% to Erythromycin, 12.5% to Clindamycin and9.37% to Cotrimoxazole.

Table 2: Antimicrobial sensitivity pattern of Gram Negative Bacilli (GNB)

Antibiotics	***Pr.mirabilis* (n=8)**		***E.coli (*n=6)**		***P.aeru* (n=6)**		***K.pneum* (n=4)**		**Citrospp (n=4)**	
Amikacin	04	50.00%	05	83.33%	04	66.66%	03	75%	03	75%
Gentamicin	05	62.5%	05	83.33%	05	83.33%	02	50%	03	75%
Cefotaxime	01	12.5%	03	50.00%	02	33.33%	01	25%	02	50%
Ceftazidime	01	12.5%	03	50.00%	02	33.33%	02	50%	02	50%
Ciprofloxacin	04	50.00%	04	66.66%	05	83.33%	03	75%	03	75%
Imipenem	08	100%	06	100%	05	83.33%	04	100%	04	100%
Piperacillin-Tazobactum	07	87.5%	05	83.33%	05	83.33%	03	75%	04	100%

In this study, among gram negative isolates, *Proteus mirabilis*,*E.coli*and*P.aeruginosa* werecommonly isolated. All gram negative bacilli except *Pseudomonas aeruginosa*which was 100%sensitive to Imipenem.

Discussion

In our study, etiological agents of Orthopaedic implant infections were identified in 70Patients (90.90%). This finding was supported by Luis Pildo*et al*[13] where the organisms was isolated in 91% of the cases. In another study done by A Hadadi positive cultures were seenin 86%[14]. A negative result does not exclude prosthesis infection. Cultures may be negativebecause of prior antimicrobial exposure, a low number of microorganism (because of adherence to the prosthesis surface), inappropriate culture media (e.g.in the case of anaerobes), or fastidiousor atypical organisms (in the case of Mycobacteria).

Out of 70 culture positive cases, aerobic gram positive cocci were isolated in 60% andaerobic gram negative bacilli in 40%. This is in accordance with the data given by Anisha fernandes[1], where she found 60.9% of gram positive cocci and 37.5% of gram negativebacilli.

Of the 70 positive cultures in this study, *Staphylococcus aureus* was the most commonpathogen isolated, 32 (45.71%), followed by CONS, 7 (10.00%) among the grampositives. *Staphylococcus aureus*, the most virulent of the many Staphylococcal species, hasdemonstrated its versatility by remaining a major cause of morbidity and mortality despite theavailability of numerous effective antistaphylococcal antibiotics. *S. aureus* is a pluripotentpathogen, causing disease through both toxin-mediated and non-toxin-mediated mechanisms. This organism is responsible for both nosocomial and community-based infections that rangefrom relatively minor skin and soft tissue infections primarily to life-threatening systemicinfections. 10% to 30% of healthy people carry *Staphylococcus aureus*, particularly inthe nose. Bed sheets, instruments and dressings have also been found to act as reservoirs[6].

Among gram negatives *Proteus mirabilis* was isolated in 11.42%,*E.coli*and *Pseudomonasaeruginosa* each in 8.57% of cases, *Klebsiella pneumonia* and *Citrobacter*spp each in 5.71%. These findings are

supported by I.Onche*et al*[15], where *Staphylococcus aureus* was seen in 44% and *Proteus* spp in 11% and alsoBirendra*et al*[16] had39.62% of *Staphylococcus aureus*in their study.

It is evident that the most effective antibiotics for the treatment of Gram-positive infections(caused mostly by *S. aureus* and Coagulase negative Staphylococci) are Linezolid and Vancomycin effective against 100% bacteria respectively.

The commonest bacteria isolated in this study, *Staphylococcus aureus* showed 100%sensitivity to Vancomycin and Linezolid, 81.25% to Amikacin and Cefotaxime, 78.12% to Ceftazidime, 56.25% to Ciprofloxacin and 15.62% to Erythromycin. Staphylococci showed highsensitivity to Vancomycin even in studies done by KhosraviA DAnisha*et al*[1].

MRSAs are resistant to b lactam antibiotic, including third generation Cephalosporins.

MRSA strains have become less susceptible to these antibiotics.

In our study, 46.87% of *Staphylococcus aureus* was found to be Methicillin resistant.,similar to study done by Trisha N. Peel *et al*[17] and studies by Trebse*et al*[18] showed36% resistance to Methicillin and 39% by Edwards [19]respectively.

The gold standard for identifying MRSA is to detect the *mecA* gene, or its product,PBP2a, by latex agglutination. However, these tests are not within the scope of many clinicallaboratories and are relatively expensive. Cefoxitin is a potent inducer of the *mec*A regulatory system. Hence, Cefoxitin is used as a surrogate marker for detection of *mec*A gene-mediatedMethicillin resistance.Cefoxitin disc is far superior to most of the currently recommendedphenotypic methods like Oxacillin disc diffusion.

The second commonest isolate CONS showed 100% sensitivity to Vancomycin andLinezolid, 71.42% to Ciprofloxacin, 57.14%to Clindamycin and Amikacin, 42.85% toErythromycin, Cefotaxime, Ceftazidime and Co-trimoxazole.

Streptococcus spp showed 100% sensitivity to most of antibiotics like Amikacin,Ciprofloxacin, Clindamycin, Erythromycin, Vancomycin and Linezolid.

Harvey Bernard opines that in the last several decades the pattern of infection has been changingand gram negative bacteria are becoming more and more common.Among gram negativeisolates, *Proteus mirabilis* was the most common isolate which showed 100% sensitivity to Imipenem, 87.5% to Piperacillin-Tazobactum, 62.5% to Gentamicin, 50% to Amikacin andCiprofloxacin, and 12.5% to Cefotaxime and Ceftazidime.

E.coli showed 100% sensitivity to Imipenem, 83.33% to Amikacin, Gentamicin andPiperacillin-Tazobactum, 66.66% to Ciprofloxacin and 50% to Cefotaxime and Ceftazidime.

Pseudomonas aeruginosa showed 83.33% sensitivity to Imipenem, Gentamicin Ciprofloxacin and Piperacillin-Tazobactum, 66.66% to Amikacin and 33.33% to Cefotaxime andCeftazidime.

Klebsiella pneumonia showed 100% sensitivity to Imipenem,75% to AmikacinCiprofloxacin and Piperacillin-Tazobactum, 50% to Gentamicin and Ceftazidime and 25% toCefotaxime.

*Citrobacter*spp showed 100% sensitivity to Imipenem and Piperacillin-Tazobactum,75% to Amikacin, Gentamicin and Ciprofloxacin, 50% to Cefotaxime and Ceftazidime.

The gram negative rods were found to be sensitive to, Piperacillin-Tazobactum,Gentamicin, Amikacin and essentially resistant to Cephalosporins tested. A findingsimilar toI. Onche[15].

Antimicrobial susceptibility test revealed horizontal spread of resistance among isolates.

Study by Anisha[1] showedmost gram negative isolates were sensitive to Carbapenems andfluoroquinolones. A finding similar to our study. The efficacy of fluoroquinolones in thetreatment of infected implants and osteomyelitis caused by Gram-negative bacilli is probably dueto: 1) their optimal diffusion into synovial fluid and bone and 2) their activity againstbiofilms.

Another study done by Ravikant das[20] showed 73.3% of all Gram-negative Enterobacteriacaewere found sensitive to combination drugs like Piperacillin + Tazobactam and Cefoperazone +

Sulbactam. Furthermore, Amikacin was found sensitive against 73.3% Gram-negativeEnterobacteriacae. Highest sensitivity with low resistance were obtained with Imipenem andCilastatin (93.3%), but they are not recommended for empirical use.

Conclusion

- Etiological agents was identified in 90.90% of infected patients.
- In the present study, aerobic Gram positive cocci were isolated in 60%,and, aerobic gramnegative bacilli in 40% of the positive cultures.
- *Staphylococcus aureus* was the most common pathogen isolated, (45.71%) followed by CONS. (10.00%) among the gram positive organisms.
- Among the isolated Gram-negative bacteria, *Proteus mirabilis* (11.42%) was the commonest pathogen, followed by *Escherichia coli* and *Pseudomonas aeruginosa* (8.57%).

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