Cross sectional analysis of aerobic bacteria and their antibiotic susceptibility pattern among orthopedic wound infections at a tertiary care hospital in Karnataka

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Abstract

Background: Orthopedic wound infections are one of the important causes of morbidity and are difficult to treat. With the development of antimicrobial resistance, the situation has further worsened and it has become difficult to manage them by conservative means leading to further deterioration in health and wellbeing of the patients. To avoid such complications, orthopedic wound infections need to be managed at the earliest by microbial isolation and appropriate antibiotic administration.

Objectives:

1. To study the profile of aerobic bacteria affecting the orthopedic wounds.
2. To study the pattern of their antibiotic susceptibility.

Methods: A Cross sectional study was conducted using secondary data of orthopedic wound infection cases maintained in the microbiology laboratory registers for a period of 1 year from September 2014 to August 2015. The bacteriological agents were isolated, identified and their antibiotic susceptibility patterns were determined using standard protocols. Analysis was done using MS Excel 2010.

Results and interpretation: Our study yielded 249 (56.08%) positive culture cases. Prevalence of Gram positive bacteria was more than Gram negative bacteria. *Staphylococcus aureus* was the predominant causative agent followed by *Pseudomonas aeruginosa.* The prevalence of MRSA was 25%. All the Gram positive cocci isolates were sensitive to linezolid and vancomycin. Members of Enterobacteriaceae family showed high sensitivity to Meropenem followed by amikacin. Polymyxin B and piperacillin-tazobactam were the most effective antibiotics among non-fermenters. All the Gram negative bacteria were resistant to penicillin and cephalosporins.

Conclusion: Microbial analysis of samples and their antibiogram is recommended for proper management of Orthopaedic wounds.

Key words: Antibiogram, Bacterial isolates, Orthopaedic wound infections

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Introduction

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 and are an important cause of morbidity and account for 70-80% mortality.[1]

A surgical wound infection can develop at any time from two to three days after surgery until the wound has healed (usually 2 to 3 weeks post operation). Occasionally, an infection can occur several months after an operation. Most surgical wound infections are limited to the skin, but can spread to deeper tissues as consequence of microbial invasion/infections, of which majority are caused by human normal flora. Most chronic infections of wounds are colonized with different microorganisms, especially problematic bacteria like antimicrobial resistant Staphylococcus aureus, Pseudomonas aeruginosa and Enterococcus, which present an increasing therapeutic challenge in wound management. It is therefore essential to specify the bacteria in surgical wounds for an individual-specific treatment.[2] Further, the problem of changes in pathogenic microbiological flora and the emergence of bacterial resistance has created major problems in the management of wound infections.[3]

Orthopaedic wound infections are one of the common causes of high morbidity and are difficult to treat. Due to the use of implants for open reduction and internal fixation, which are foreign bodies to the body, orthopaedic wounds are at increased risk of microbiological contamination and infection.[3] Once frank infection develops the management becomes primarily surgical with repeated debridement. Not only it is a physical ailment for the patient, but also adds to his psychological and financial load. The patient, if untreated or irrationally treated goes in for serious life-threatening and limb-threatening consequences and may also land up in an emotional breakdown.[4] A regular bacteriological review of infected wounds is therefore a necessity if affected patients must receive qualitative health care. With this background, the following study was conducted to find out the various organisms causing orthopaedic wound infection and to identify the antibiotic susceptibility pattern of the isolated organisms.[1]

Materials and Methods

A cross sectional study was conducted in the Microbiology department of a private tertiary care hospital in Shimoga district of Karnataka. Prior permission for the study was obtained from concerned authorities. Secondary data of orthopedic wound infection cases maintained in the laboratory registers of microbiology department for a period of 1 year from September 2014 to august 2015 were collected for the study. The following information were noted –name, age, sex, case history, pre-operative antibiotics used, organism isolated and their antibiotic susceptibility. Analysis was done using MS Excel 2010.

All cases of orthopedic wound infections at this institute are routinely sent for microbiological analysis. The samples in the laboratory will be processed for direct microscopy, aerobic culture and sensitivity as per the standard protocol. The samples will be inoculated on to Nutrient agar (NA), Mac Conkey Agar (MA) and Blood Agar (BA) plates and incubated at 37^0C for 24 hours aerobically. After incubation, identification of bacteria from positive cultures will be done with a standard microbiological technique which includes studying the colonial morphology, Gram stain and biochemical reactions.[5] The antibiotic sensitivity testing of all isolates will be performed by modified Kirby-Bauer's disc diffusion method on Mueller Hinton agar using antibiotics as per CLSI guidelines.[6] The following drugs were tested.

For Gram positive cocci- Penicillin (10units), Erythromycin (15µg), Clindamycin (2µg), Ciprofloxacin (5µg), Cotrimoxazole (25µg), Chloramphenicol (30µg), Gentamicin (10µg), Linezolid 310µg), Vancomycin (30µg), Teicoplanin (30µg), Tetracycline (30µg). High level gentamycin (120 µg) in case of Enterococcus.

For Gram negative bacilli- Ampicillin (10µg), Amoxyclavulanic acid (30 µg), Cotrimoxazole (25 µg), Tetracycline (30 µg), Chloramphenicol (30 µg), Gentamicin (10 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Cefepime(30 µg), Ceftriaxone(30µg), Cephotaxime(30 µg), Ceftazidime (30 µg), Cefazoline (30 µg), Cefuroxime (30 µg), Aztreonam (30µg), Piperacillin (100 µg), Meropenem (10 µg).

For Non-fermenters- Ampicillin (10 µg), Gentamicin (10 µg), Amoxyclavulanic acid (30 µg), Amikacin (30 µg), Ciprofloxacin (5 µg), Cefoxitin (30 µg), Cefepime (30 µg), Ceftriaxone (30 µg), Cephotaxime (30 µg), Ceftazidime (30 µg), Cefazoline (30 µg), Cefuroxime (30 µg), Aztreonam (30 µg), Piperacillin (100 µg), Meropenem (10 µg), Levofloxacin (5 µg), Ticarcillin (75 µg), Tobramycin (10 µg), Piperacillin-Tazobactam (100/10 µg), Polymyxin B (30 units).

Results

Out of 444 pus samples received for culture and sensitivity in the microbiology laboratory, 249 (56.08%) cases yielded positive culture while 195 (43.91%) cases had no growth.

Among the 249 culture positive cases majority i.e. 180 (72.28%) were males and 69 (27.71%) were females yielding a male: female ratio of 1:0.38. The cases were more in the age group of 41-50 years i.e.60 (24.09%) followed by 57 (22.89%) cases in the age group of 31-40 years as shown in **Table 1**.

Among the 249 culture positive pus samples, 204 (81.92%) yielded pure bacterial isolates and 45 (18.07%) yielded mixed infections; so a total number of 294 organisms were isolated from 249 pus samples. Among the 294 organisms isolated from culture positive pus samples, 165 (56.12%) were Gram positive cocci and 129 (43.87%) were Gram negative bacilli.

Fig. 1 shows the bacteriological profile of infected wound sample. Out of Gram positive cocci isolates, *Staphylococcus aureus* 99 (33.67%) was the commonest organism followed by *Coagulase negative Staphylococci* - 33 (11.22%), *Enterococcus fecalis* 24 (8.16%) and *Streptococcus pyogenes* 9 (3.06%). Amongst Gram negative bacilli, *Pseudomonas aeruginosa* 42 (14.28%) was the most common organism followed by *Escherichia coli* 24 (8.16%), *Klebsiella pneumoniae* 18 (6.12%), Acinetobacter species 15 (5.1), *Enterobacter aerogenes* 18 (6.12%) and *Proteus mirabilis* 12 (4.08%).This showed that Gram positive *Staphylococcus aureus* is the most common causative agent of orthopaedic wound infection followed by Gram negative *Pseudomonas aeruginosa*.

All the GPC isolates were sensitive to linezolid and vancomycin. Meropenem and amikacin were the most effective drugs among enterobacteriaceae species and Polymyxin B and piperacillin-tazobactam among non-fermenters. All the Gram negative bacteria were resistant to penicillin and cephalosporins.

Among the *Staphylococcus aureus* isolates majority i.e. 96 (96.96) were sensitive to tetracycline followed by chloramphenicol 93 (93.93%), teicoplanin 84 (84.84%) and gentamicin 72 (72.72%). Most of the *Coagulase Negative Staphylococcus* isolates 27 (81.81%) were sensitive to teicoplanin, whereas all were sensitive to chloramphenicol and tetracycline 96 (96.96%). Majority of the *Streptococcus pyogenes* were sensitive to all the antibiotics tested. Among Enterococcus 18 (75%) were sensitive to chloramphenicol, high level gentamicin, ciprofloxacin, cotrimoxazole and tetracycline as presented in **Table 2.**

Among the Staphylococcus species, Methicillin Sensitive *Staphylococcus aureus* (MSSA) were 50%, Methicillin resistant *Staphylococcus aureus* (MRSA) were 25%, Methicillin Sensitive *Coagulase Negative Staphylococcus* (MSCONS) were 15.9% and Methicillin Resistant *Coagulase Negative Staphylococcus* (MRCONS) were 9.09%. **(Table 3)**

Table 4 shows all the isolates of *Escherichia coli* were sensitive to chloramphenicol and amikacin. Majority of them were sensitive to tetracycline 21 (87.5%) followed by cotrimoxazole, gentamicin and meropenem 15 (62.5%) each. All the isolates of *Klebsiella pneumoniae* were sensitive to meropenem whereas 15 (83.33%) were sensitive to tetracycline and chloramphenicol and 12 (66.66%) to cotrimoxazole, gentamicin, amikacin and ciprofloxacin each. Majority i.e 15(83.33%) of the Enterobacter species were sensitive to meropenem and amikacin each and 12 (66.66%) were sensitive to tetracycline and gentamycin. All the isolates of *Proteus mirabilis* were sensitive to meropenem, gentamicin and amikacin and 9 (75%) were sensitive to ciprofloxacin.

Pseudomonas aeruginosa showed maximum sensitivity to Polymyxin B 39 (92.85) followed by piperacillin –tazobactam 30 (71.42%), meropenem 24 (57.14%) and amikacin 21 (50%). Majority of acinetobacter species were sensitive to Polymyxin B 13 (86.66%) followed by piperacillin –tazobactam 12 (80%) and meropenem, amikacin 9 (50%) each.**(Table 5)**

Table 1: Age and sex distribution of culture positive samples

Age in Years	**Male**		**Female**		**Total**	
	No.	**(%)**	**No.**	**(%)**	**No.**	**(%)**
0-10	6	(3.33)	6	(8.69)	12	(4.81)
11-20	3	(1.66)	3	(4.34)	6	(2.4)
21-30	24	(13.33)	6	(8.69)	30	(12.04)
31-40	54	(30)	3	(4.34)	57	(22.89)
41-50	33	(18.33)	27	(39.13)	60	(24.09)
51-60	30	(16.66)	12	(17.39)	42	(16.86)
61-70	12	(6.66)	0	(0)	12	(4.81)
71-80	18	(10)	12	(17.39)	30	(12.04)
Total	180	(100)	69	(100)	249	(100)

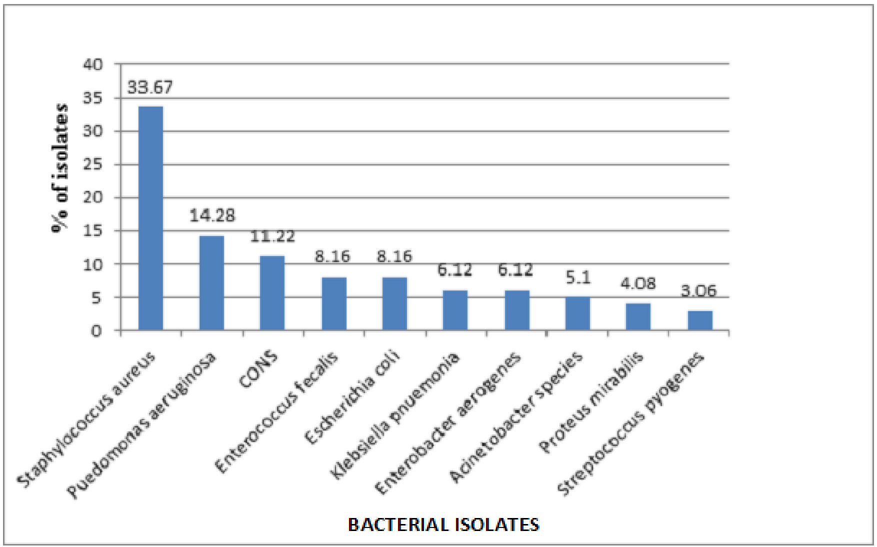

Fig. 1: Bacteriological profile of infected wound samples

Table 2: Antibiotic Sensitivity Pattern of GPC (n=165)

	S. aureus (99)		CONS (33)		Strep. Pyogenes (9)		Enterococcus faecalis(24)	
	NO.	(%)	NO.	(%)	NO.	(%)	NO.	(%)
Penicillin	6	(6.06)	9	(27.27)	6	(66.66)	6	(25)
Erythromycin	57	(57.57)	21	(63.63)	6	(66.66)	12	(50)
Clindamycin	54	(54.54)	24	(72.72)	6	(66.66)	12	(50)
Ciprofloxacin	30	(30.3)	18	(54.54)	7	(77.77)	18	(75)
Cotrimoxazole	42	(42.42)	9	(27.27)	7	(77.77)	18	(75)
Chloramphenicol	93	(93.93)	33	(100)	6	(66.66)	18	(75)
Gentamicin	72	(72.72)	18	(54.54)	6	(66.66)	18 *	(75)
Linezolid	99	(100)	33	(100)	9	(100)	24	(100)
Vancamycin	99	(100)	33	(100)	9	(100)	24	(100)
Teicoplanin	84	(84.84)	27	(81.81)	6	(66.66)	18	(75)
Tetracycline	96	(96.96)	30	(100)	6	(66.66)	18	(75)

CONS- Coagulase negative Staphylococci
Strep. pyogenes- Streptococcus pyogenes
* Tested for high level gentamycin

Table 3: Susceptibility pattern of MRSA, MSSA, MRCONS and MSCONS

Antibiotics	MSSA 66 (50%)		MRSA 33 (25%)		MS CONS 15.9 (21%)		MR CONS 12 (9.09%)	
	NO.	(%)	NO.	(%)	NO.	(%)	NO.	(%)
Penicillin	6	(9.09)	0	(0)	9	(42.85)	0	(0)
Erythromycin	48	(72.72)	9	(27.27)	12	(57.14)	9	(75)
Clindanycin	48	(72.72)	6	(18.18)	15	(71.42)	9	(75)
Tetracycline	66	(100)	30	(90.9)	21	(100)	9	(75)
Chloramphenicol	66	(100)	27	(81.81)	21	(100)	12	(100)
Cotrimoxazole	27	(40.9)	15	(45.45)	6	(28.57)	3	(25)
Ciprofloxacin	15	(22.7)	15	(45.45)	15	(71.42)	3	(25)
Gentamicin	54	(81.81)	18	(54.54)	15	(71.42)	6	(50)
Linezolid	66	(100)	33	(100)	21	(100)	12	(100)
Vancamycin	66	(100)	33	(100)	21	(100)	12	(100)
Teicolplanin	54	(81.81)	30	(90.9)	15	(71.42)	12	(100)
Rifampicin	63	(95.45)	30	(90.9)	21	(100)	12	(100)

MSSA- Methicillin Sensitive Staphylococcus aureus
MRSA- Methicillin resistant Staphylococcus aureus
MSCONS- Methicillin sensitive Coagulase negative Staphylococci
MRCONS- Methicillin resistant Coagulase negative Staphylococci

Table 4: Antibiotic sensitivity pattern of Enterobacteriaceae (n=72)

	Enterobacteriaceae N=72			
	E. coli (24)	Klebsiella (18)	Enterobacter (18)	Proteus (12)
Antibiotics	NO. (%)	NO. (%)	NO. (%)	NO. (%)
Ampicillin	4 (16.66)	3 (16.66)	1 (5.55)	3 (25)
Amoxyclavulanic acid	6 (25)	3 (16.66)	1 (5.55)	3 (25)
Cotrimoxazole	15 (62.5)	12 (66.66)	1 (5.55)	6 (50)
Tetracycline	21 (87.5)	15 (83.33)	12 (66.66)	6 (50)
Chloramphenicol	24 (100)	15 (83.33)	9 (50)	6 (50)
Gentamicin	15 (62.5)	12 (66.66)	12 (66.66)	12 (100)
Amikacin	24 (100)	12 (66.66)	15 (83.33)	12 (100)
Ciprofloxacin	3 (12.5)	12 (66.66)	6 (33.33)	9 (75)
Cefoxitin	12 (50)	6 (33.33)	3 (16.66)	6 (50)
Cefepime	6 (25)	3 (16.66)	3 (16.66)	6 (50)
Ceftriaxone	3 (12.5)	3 (16.66)	3 (16.66)	6 (50)
Cephotaxime	3 (12.5)	3 (16.66)	3 (16.66)	6 (50)
Ceftazidime	3 (12.5)	3 (16.66)	3 (16.66)	6 (50)
Cefazoline	3 (12.5)	3 (16.66)	3 (16.66)	6 (50)
Cefuroxime	3 (12.5)	3 (16.66)	3 (16.66)	6 (50)
Aztreonam	3 (12.5)	3 (16.66)	3 (16.66)	6 (50)
Piperacillin	6 (25)	3 (16.66)	1 (5.55)	6 (50)
Meropenem	15 (62.5)	18 (100)	15 (83.33)	12 (100)

E. coli- Escherichia coli

Table 5: Antibiotic Sensitivity Pattern of Non fermenters (n=57)

	Pseudomonas N=42	Acinetobacter N=15
Antibiotics	NO. (%)	NO. (%)
Ampicillin	0 (0)	0 (0)
Amoxyclavulanic acid	0 (0)	0 (0)
Gentamicin	9 (21.42)	6 (40)
Amikacin	21 (50)	9 (60)
Ciprofloxacin	9 (21.42)	6 (40)
Cefoxitin	0 (0)	1 (6.66)
Cefepime	6 (14.28)	1 (6.66)
Ceftriaxone	3 (7.14)	1 (6.66)
Cephotaxime	3 (7.14)	1 (6.66)
Ceftazidime	3 (7.14)	1 (6.66)
Cefazoline	12 (28.57)	1 (6.66)
Cefuroxime	9 (21.42)	1 (6.66)
Aztreonam	3 (7.14)	2 (13.33)
Piperacillin	12 (28.57)	1 (6.66)
Meropenem	24 (57.14)	9 (60)
Levofloxacin	12 (28.57)	3 (20)
Ticarcillin	18 (42.85)	6 (40)
Tobramycin	21 (50)	7 (46.66)
Piperacillin-Tazobactam	30 (71.42)	12 (80)
Polymyxin B	39 (92.85)	13 (86.66)

Discussion

In our study 249 (56.08%) cases yielded positive culture while 195 (43.91%) cases showed no growth. Similar trend was reported by some other studies.[1,7,8,9,10] However, few others have found out very high proportion of culture positive cases in their studies contrary to our findings.[3,11,12,13]

Higher male distribution of culture positive cases was seen in our study which corroborates with the other studies.[3,8, 9,12,14] This may be due to the fact that men are more prone for trauma because of their outdoor activities. Maximum number of cases belonged to age group 41-50 years followed by 31-40 which differs from other studies where maximum belonged to age group 30-40 years.[3,9]

The prevalence of MRSA was 25% which was more or less similar to the other studies.[8,15,16] On the contrary Amatya J et al. reported very low prevalence of only 2.2%.[9] Prevalence of Gram positive bacteria was more than Gram negative bacteria in our study which was contrary to other studies.[1,3,7,1,17]

Microbiological profile of wound infection shows that *Staphylococcus aureus* was the predominant causative agent which corroborated with other studies.[1,3, 4,8,10,12,13,15,16,18,19] However, few studies found *Pseudomonas aeruginosa*[15,16] as the second most common causative organism similar to our study while some others found *Escherichia coli* [4,10,13] contrary to our study. Further some other authors showed that *Pseudomonas aeruginosa* had highest incidence followed by *Staphylococcus aureus* in contrast to our findings.[9,11,20,21,22]

In our study the members of Enterobacteriaceae family showed high sensitivity to Meropenam followed by amikacin. This correlates with other studies.[3,9,12] However some other studies revealed amikacin as the most effective drug[13,15] while some other reported imipenem as most effective antibiotic.[1,9,18,23]

Our study revealed Polymyxin B and piperacillin-tazobactam was the most effective antibiotic among non fermenters. Our finding correlated with a study conducted by Shanmugam P et al. [24] where 100% non-fermenters were sensitive to Polymyxin B. Some studies revealed piperacillin- tazobactam as the most effective drug.[3,4] The findings from the study conducted by Jain V et al. did not correlate with our study findings which reported that among the non-fermenters the most sensitive drugs were Imipenam followed by Polymyxin B and Colistin.[1]

All the Gram positive cocci isolates in our study were sensitive to linezolid and vancomycin which was concosance with other studies.[1,3,4,12,25] While some other studies revealed genatmicin as the most effective antibiotic.[13,15] *Staphylococcus aureus* showed the highest sensitivity to Linezolid which correlated with other studies.[21,25] Enterococci isolates were sensitive to Linezolid which was concosance with other studies.[21,26,27] *Coagulase Negative Staphylococcus* isolates were found to be susceptible to Linezolid with correlated with other studies.[17,25]

In our study all the Gram negative bacteria showed resistance to penicillin and cephalosporins group which was concordant with the study which showed Cefaperazone and ceftriaxone to be the most effective antibiotics.[7]

Conclusion

Orthopaedic wound infections are caused by wide variety of drug resistant microorganisms. As a result, it becomes necessary for routine microbial analysis of samples and their antibiogram for their proper management.

Conflicts of Interest: None declared

Source of Support: Nil

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