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ANTIBIOTIC RESISTANCE PATTERN IN BACTERIA ISOLATED FROM ORTHOPAEDIC RELATED INFECTIONS

Miss. Nikita Sundrani	Postgraduate student, People's College of Medical Science & Research Center, Bhopal, MP
Dr. Kalpana Sadawarte*	Professor & Head, Department of Microbiology, People's College of Medical Science & Research Center, Bhopal, MP*Corresponding Author
Dr. Harish Rao	Professor & Head, Department of Orthopaedics, People's College of Medical Science & Research Center, Bhopal, MP
Mr. Tukaram Prabhu	Assistant Professor, Department of Microbiology, People's College of Medical Science & Research Center, Bhopal, MP

ABSTRACT **Background.** Orthopaedics & Implant related infections continue to pose a problem for the orthopaedicians. An increase in the number of multidrug resistant bacteria stresses the value of an adequate diagnosis, leading to a proper therapy of these patients. **Objective.** To isolate bacteria causing orthopaedic related infections and determine their antibiotic resistance pattern. **Materials & methods**. A total of 111 samples were received during the study period in the Department of Microbiology & were processed. Bacteria causing orthopaedic related infections were isolated and their antimicrobial susceptibility testing was determined. **Results**. There were 66 (59%) isolates that showed culture positivity & were processed. Among which, gram positive cocci were 35 (53%) & gram negative bacilli were 31 (47%). The common gram positive cocci were Methicillin sensitive Staphylococcus aureus 31.81% followed by CoNS 10.6% & MRSA 10.6% While the common gram negative bacilli were Pseudomonas aeruginosa 18.18%, Klebsiella pneumoniae 12.12%, Escherichia coli 7.5%, Citrobacter freundii 4.5%, Proteus mirabilis 3.03% & Acinetobacter baumannii 1.51%. Most of the gram positive cocci were resistance to the following antibiotics- ciprofloxacin, penicillin, erythromycin. Most of the gram negative bacilli were resistance to the cephalosporins group of drugs mainly ceftriaxone & ceftazidime **Conclusion**. Gram positive cocci were the commonest isolates from orthopaedic related infection as compare to gram negative bacteria. Antibiotic resistance pattern should be given adequate stress so as to get better results regarding orthopaedic related infections.

KEYWORDS : Antimicrobial susceptibility testing, Orthopaedic infections, Gram staining, multidrug-resistant bacteria

INTRODUCTION

One of the problem that affects millions of people worldwide are the bone, joint degenerative & inflammatory problems. In developed countries, in people over 50 years of age, these problems account for half of all chronic diseases.[1] Bones and joints are normally termed as sterile areas. Bacteria may reach the sites by hematogenous spread or extrinsic and intrinsic contiguous focus of infection. Osteomyelitis is termed as an infection of the bone. Osteomyelitis was quoted by Charaka and Sushruta in their Sanskrit treatises.[2][3] Hippocrates [500-400 BC] described bone diseases with injury as the susceptible factor. Healing and Non-healing factors of bone diseases were also described by him. Nelton devised the term osteomyelitis in 1834. Osteomyelitis leads to bone necrosis, inflammatory destruction of bone, and the formation of new bones.

Resistance of bacterial pathogens to frequently used antibiotics and the unfolding of multidrug-resistant bacteria is a worldwide challenge that is increasing at an intimidating rate, which has led antibiotic options to become both limited and costly. Despite innumerable actions taken to tackle antibiotic resistance, global trends show no hints of slowing down. As a consequence, infections with these resistant bacteria will lead the way to more serious illnesses, treatment failures, prolonged hospital admissions, and a rise in healthcare cost.[4]

Orthopaedic related infections are the infections of the musculoskeletal system. Due to great number of total hip & knee arthroplasties that are being performed worldwide, these infections represent a serious problem.[5] Though artificial joints can remarkably improve the quality of life of patients, but as a consequence, non-fulfilment or non-success of prosthetics can open on to high suffering & morbidness.

In view of the challenge, antimicrobial resistance poses a challenge to healthcare systems worldwide, which is linked with the results and implications of postoperative orthopaedic infections. This study was focused to find the antibiotic resistance patterns of bacteria isolated from orthopaedic infections.[4]

MATERIAL & METHOD

The study was conducted in the Department of Microbiology of People's college of Medical Sciences, RC & Hospital after obtaining clearance from Institutional Ethics Committee. It was a Cross-sectional and observational study. Direct Microscopy was done on the samples received from the orthopaedics department by the Gram Staining Method.[6] Then Culture was performed on Blood agar and MacConkey agar as per standard methods. For identification of bacteria, biochemical tests were performed that includes catalase test, coagulase test, TSI, urease test, oxidase test, motility test etc as per standard methods.[6][7] Then Antimicrobial Susceptibility Testing was performed on Mueller Hinton agar by Kirby- Bauer Disc Diffusion Method[6] as per Clinical and Laboratory Standard Institute (CLSI) 2022 guidelines, to get the antibiotic resistance pattern.

RESULTS

A total of 111 samples were received during the study period & were processed. Result was presented as Mean ± Standard Deviation. Culture positive were 59% and culture negative were 41% from total samples of 111. Among the total isolates 66 (100%), gram positive cocci were 35 (53%) & gram negative bacilli were 31 (47%). More gram positive cocci were isolated as compare to gram negative bacilli. Methicillin sensitive Staphylococcus aureus were 31.81% followed by CoNS 10.6% & MRSA 10.6%. While the common gram negative bacilli isolated were Pseudomonas aeruginosa 18.18%, Klebsiella pneumoniae 12.12%, Escherichia coli 7.5%, Citrobacter freundii 4.5%, Proteus mirabilis 3.03 & Acinetobacter baumannii 1.51%.

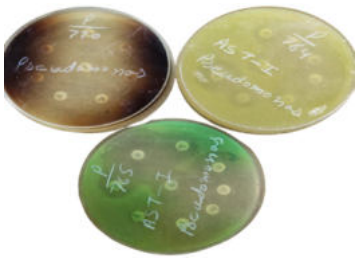


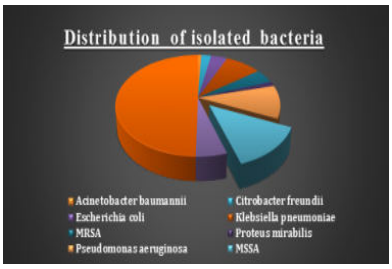
Fig. 1 Growth of pigmented colonies of Pseudomonas aeruginosa on MHA

Table 1 – Frequency & percentage of isolated bacteria

Organism	Frequency	Percent
MSSA	21	31.81
Pseudomonas aeruginosa	12	18.18
Klebsiella pneumoniae	8	12.12
MRSA	7	10.6
CoNS	7	10.6
Escherichia coli	5	7.5
Citrobacter freundii	3	4.5
Proteus mirabilis	2	3.03
Acinetobacter baumannii	1	1.51
Total	66	100

MSSA: Methicillin sensitive Staphylococcus aureus, MRSA: Methicillin resistant Staphylococcus aureus ,CoNS: Coagulase negative Staphylococcus aureus

Chart 1. Distribution of isolated bacteria



Most of the gram positive cocci were resistance to the following antibiotics- ciprofloxacin, penicillin, erythromycin. Most of the gram negative bacilli were resistance to the following antibiotics cephalosporins group of drugs mainly ceftriaxone & ceftazidime. Multi-drug resistance were more common with Methicillin resistant Staphylococcus aureus, Klebsiella pneumoniae, Proteus mirabilis & Acinetobacter baumanii.

Statistical analysis using chi square test was done. The value was found to be statistically significant ($p<0.001$) AST pattern is depicted in the following tables

Table 2 - AST Pattern of gram positive cocci

	MSSA		MRSA		CoNS	
	R	S	R	S	R	S
Antibiotics	Total = 21 (100%)		Total = 7(100%)		Total = 7 (100%)	
Penicillin	15 (71.43%)	6 (28.57%)	4 (57.15%)	3 (42.85%)	6 (85.71%)	1 (14.29%)
Cefoxitin	0(0%)	21 (100%)	7 (100%)	0 (0%)	3 (42.85%)	4 (57.15%)
Ampicillin/sulbactum	5 (23.81%)	16 (76.19%)	6 (85.71%)	1 (14.29%)	2 (28.58%)	5 (71.42%)
Amoxy Clavunate	13 (61.90%)	8 (38.10%)	4 (57.15%)	3 (42.85%)	4 (57.15%)	3 (42.85%)
Vancomycin	3 (14.29%)	18 (85.71%)	2 (28.58%)	5 (71.42%)	5 (71.42%)	2 (28.58%)
Gentamicin	4 (19.04%)	17 (80.95%)	3 (42.85%)	4 (57.15%)	1 (14.29%)	6 (85.71%)
Doxycycline	3 (14.29%)	18 (85.71%)	1 (14.29%)	6 (85.71%)	3 (42.85%)	4 (57.15%)
Chloremphenicol	2 (9.53%)	19 (90.47%)	5 (71.42%)	2 (28.58%)	1 (14.29%)	6 (85.71%)
Erythromycin	8 (38.10%)	13 (61.90%)	6 (85.71%)	1 (14.29%)	4 (57.15%)	3 (42.85%)
Clindamycin	5 (23.81%)	16 (76.19%)	5 (71.42%)	2 (28.58%)	2 (28.58%)	5 (71.42%)
Linezolid	4 (19.04%)	17 (80.95%)	1 (14.29%)	6 (85.71%)	1 (14.29%)	6 (85.71%)
Ciprofloxacin	10 (47.62%)	11 (52.38%)	6(85.71%)	1 (14.29%)	2 (28.58%)	5 (71.42%)
Levofloxacin	6 (28.57%)	15 (71.43%)	3 (42.85%)	4 (57.15%)	1 (14.29%)	6 (85.71%)
Co-trimoxazole	7 (33.33%)	14 (66.67%)	5 (71.42%)	2 (28.58%)	2 (28.58%)	5 (71.42%)

MSSA: Methicillin sensitive Staphylococcus aureus, MRSA: Methicillin resistant Staphylococcus aureus CoNS: Coagulase negative Staphylococcus aureus

Table 3 - AST Pattern of gram negative bacteria

	Klebsiella pneumoniae		Escherichia coli		Citrobacter freundii		Proteus mirabilis	
	R	S	R	S	R	S	R	S
Antibiotics	Total = 8 (100%)		Total = 5 (100%)		Total = 3 (100%)		Total = 2 (100%)	
Cefoxitin	6 (75%)	2 (25%)	1 (20%)	4 (80%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Cefuroxime	7 (87.5%)	1 (12.5%)	3 (60%)	2 (40%)	1 (33.33%)	2 (66.67%)	2 (100%)	0 (0%)
Ceftriaxone	6 (75%)	2 (25%)	4 (80%)	1 (20%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Ceftazidime	7 (87.5%)	1 (12.5%)	1 (20%)	4 (80%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Cefepime	4 (50%)	4 (50%)	2 (40%)	3 (60%)	1 (33.33%)	2 (66.67%)	1 (50%)	1 (50%)
Ampicillin/ sulbactum	6 (75%)	2 (25%)	3 (60%)	2 (40%)	1 (33.33%)	2 (66.67%)	1 (50%)	1 (50%)
Amoxy Clavunate	5 (62.5%)	3 (37.5%)	4 (80%)	1 (20%)	1 (33.33%)	2 (66.67%)	1 (50%)	1 (50%)
Piperacillin tazobactum	2 (25%)	6 (75%)	2 (40%)	3 (60%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Ertapenem	4 (50%)	4 (50%)	1 (20%)	4 (80%)	1 (33.33%)	2 (66.67%)	2 (100%)	0 (0%)
Gentamicin	5 (62.5%)	3 (37.5%)	3 (60%)	2 (40%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Amikacin	4 (50%)	4 (50%)	1 (20%)	4 (80%)	1 (33.33%)	2 (66.67%)	1 (50%)	1 (50%)
Chloremphenicol	1 (12.5%)	7 (87.5%)	2 (40%)	3 (60%)	1 (33.33%)	2 (66.67%)	0 (0%)	2 (100%)
Ciprofloxacin	6 (75%)	2 (25%)	4 (80%)	1 (20%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Levofloxacin	5 (62.5%)	3 (37.5%)	1 (20%)	4 (80%)	2 (66.67%)	1 (33.33%)	1 (50%)	1 (50%)
Co-trimoxazole	7 (87.5%)	1 (12.5%)	2 (40%)	3 (60%)	1 (33.33%)	2 (66.67%)	1 (50%)	1 (50%)

Table 4 – AST Pattern of Non-fermenters

Antibiotics	Pseudomonas aeruginosa		Acinetobacter baumannii	
	R	S	R	S
	Total = 12(100%)		Total =1 (100%)	
Ceftazidime	1 (8.33%)	11 (91.67%)	1 (100%)	0 (0%)
Cefepime	2 (16.67%)	10 (83.33%)	0 (0%)	1 (100%)
Ampicillin/sulbactum	4 (33.33%)	8 (66.67%)	1 (100%)	0 (0%)
Piperacillin tazobactum	2 (16.67%)	10 (83.33%)	0 (0%)	1 (100%)
Meropenem	3 (25%)	9 (75%)	1 (100%)	0 (0%)
Gentamicin	4 (33.33%)	8 (66.67%)	1 (100%)	0 (0%)
Amikacin	1 (8.33%)	11 (91.67%)	1 (100%)	0 (0%)
Ciprofloxacin	5 (41.67%)	7 (58.33%)	0 (0%)	1 (100%)
Levofloxacin	8 (66.67%)	4 (33.33%)	0 (0%)	1 (100%)

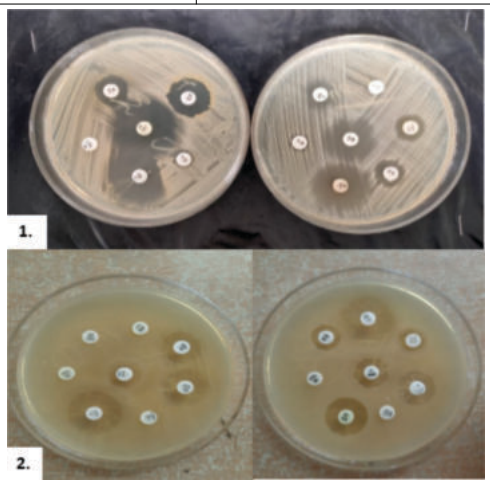


Fig.2 (left)
1. Antimicrobial susceptibility testing for gram positive cocci
2. Antimicrobial susceptibility testing for gram negative bacilli

Fig.3 (left) Antimicrobial susceptibility testing for Pseudomonas aeruginosa

DISCUSSION

Orthopaedic related infections remains a major obstacle in modern trauma and orthopaedic surgery. Despite best practices in medical and surgical management, neither prophylaxis nor treatment of orthopaedic related infections is successful in all cases, and can lead to infections that negatively impact clinical outcome and remarkably increase healthcare expense. Pre-operative and correctly-timed prophylactic antibiotic intervention is compulsory for a majority of orthopaedic procedure.[8]

Among the 111 samples, 59% (66) of the samples showed culture positivity, comprising of both Gram-positive cocci and Gram negative bacilli and 41% (45) samples were culture negative. This results were well correlating with Sujata Prasad et al[9] showing the growth rate of > 50 % and Marta Ribeiro et al[10] also showing the growth rate around 55-60%.

In this present study, among the organisms isolated, 53% were Gram-positive cocci and 47% were gram-negative bacilli. This is corresponding to Dorota Teterycz et al[11] where the gram-positive organism accounts for 66% and Morrad mohammed et al[12] where gram negative bacilli accounts for 33%. Nearly two-third of orthopaedics related infections are caused by gram positive cocci especially Staphylococcus aureus and CoNS. In this study, among the Staphylococcus aureus isolated, 75% were MSSA (Methicillin sensitive Staphylococcus aureus) and 25% were MRSA (Methicillin resistant Staphylococcus aureus). This corresponds to the study by Dorota Teterycz et al [11] where he says that more than 60% will be MSSA (Methicillin sensitive Staphylococcus aureus) and Morrad Mohammad et al[12] says that MRSA (Methicillin resistant Staphylococcus aureus) are more than 39% and A.S Haddadin et al[13] also states that MRSA (Methicillin resistant Staphylococcus aureus) are nearly 30%.

Most of which highlighted that Sulfamides and Glycopeptides for the major Gram-positive strains and Fluoroquinolones, Carbapenems, and Aminoglycosides for the most represented Gram-negative isolates could be the most suitable therapeutic choice for most multi-drug resistance isolates.[14]

In almost similar accordance with our study, in the study done by Gemedo Misha et al[15] , antibiotic resistance profiles were reported for the organisms isolated from surgical site of infected patients where he showed that the Gram-positive pathogens showed high resistance towards penicillin (66.67%), erythromycin (66.67%), and clindamycin (66.67%). And the Gram-negative pathogens showed high resistance towards cefepime (87.88%), ceftriaxone (78.79%), cefuroxime (63.63%), cotrimoxazole (54.55%), ciprofoxacin (60.60%), and ampicillin (60.60%).

CONCLUSION

Thus, it can be said that orthopaedic related infections continue to create a problem for the orthopaedicians. The diagnosis and cure of these infections are complicated by an increase in the number of multidrug-resistant bacteria stressing the value of a required diagnosis, leading to a proper remedy of these cases.

Ethical consideration: Approval was obtained from the Institutional Ethics Committee (reg. no. ECR/519/Inst/MP/2014/RR-20)

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