

ORIGINAL RESEARCH

A Study of Aerobic Bacteriological Profile and Antimicrobial Susceptibility Pattern of Isolates in Patients with Chronic Osteomyelitis at Tertiary Care Hospital

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ABSTRACT

Introduction: In recent times, there has been change in bacteriological profile of chronic osteomyelitis cases as well as susceptibility of antibiotics.

Objectives: 1. To study Demographic characteristics of Chronic Osteomyelitis cases, 2. To isolate causative Aerobic bacteria (other than Mycobacteria) 3. To analyze Antimicrobial Susceptibility Pattern of isolated aerobic bacteria.

Methodology: A cross sectional hospital-based study of 1 year duration was conducted at Department of Microbiology, S. P. Medical College, Bikaner, Rajasthan. After obtaining informed verbal consent, 110 chronic osteomyelitis cases were recruited to study following nonprobability consecutive sampling. Their pus samples were taken and studied for staining, presence of enzymes, biochemical reactions, fermentation tests, antibiotic susceptibility testing. Obtained results were analysed and presented through tables and diagrams.

Results: 50% cases had *S. aureus* and 18% had *P. aeruginosa* in their pus samples. Majority of patients were male and almost 1/3rd cases belonged to 16-30 years age group. *S. aureus* isolates were sensitive to Cotrimoxazole & Linezolid. Most of the isolate of *Klebsiella pneumoniae* (83.33%) were sensitive to Meropenem. All isolates of *Pseudomonas aeruginosa* (100%) were sensitive to Polymyxin B and Colistin C.

Conclusion: Study of bacteriological profile and antimicrobial susceptibility pattern helps significantly in clinical management decisions.

Keywords: Osteomyelitis, Aerobic Bacteria, Antimicrobial Susceptibility.

INTRODUCTION

The incidence of osteomyelitis has been lowered to a certain extent due to the rapid diagnosis and the availability of multiple antibiotics along with modern treatment facilities;^[1] but still, osteomyelitis is an ongoing problem due to emergence of multi drug resistant strains among bacterial pathogens like *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Inappropriate

and excessive use of antibiotics is considered as the main cause of development of drug resistance.^[2]

In all cases of osteomyelitis including chronic osteomyelitis most commonly isolated micro-organism is *Staphylococcus aureus*.^{[3], [4], [5], [6]} Other bacteria causing chronic osteomyelitis are *Pseudomonas spp*, *E.coli*, *Proteus spp*, *Klebsiella spp*, *Coagulase negative Staphylococci*, *Enterococcus spp*, *Enterobacter spp*, *Acinetobacter spp*, anaerobes like *Pepto-streptococcus spp*, *Bacteroides spp*, *Clostridium spp*.^[7] The infection generally is due to a single organism, but polymicrobial infections can occur, especially in the diabetic foot^[8]. But there has been a change in trend of profile of causative organisms with gram negative bacilli emerging as significant causative agent in recent times.^[8]

These circumstances mandate the need to isolate causative agent and to know its antimicrobial susceptibility pattern from each and every such case to target and treat infection with precision instead of blindly prescribing antibiotics and waiting for the result thus delaying the process of healing in already a chronic and debilitating illness. The key to successful management is early diagnosis and appropriate surgical and antimicrobial treatment. A multidisciplinary approach is required, involving an orthopedic surgeon and an infectious disease specialist^[8].

Hence, this study was undertaken in an effort to assist clinicians in the effective management of this condition and provide an antibiogram for starting of proper empiric therapy as long as the results of culture and sensitivity would be obtained to reduce cost and period of treatment of these patients.

OBJECTIVES

1. To study demographic characteristics of chronic osteomyelitis cases
2. To isolate and identify causative Aerobic bacteria (other than Mycobacteria) from pus discharge obtained from cases of chronic osteomyelitis.
3. To study and analyze the pattern of antimicrobial susceptibility of isolated bacteria from cases of chronic osteomyelitis.

METHODOLOGY

This study was a cross sectional study conducted at Department of Microbiology, Sardar Patel Medical College, Bikaner and samples were collected from P.B.M. Hospital, Bikaner from Jan 2021 to Dec 2021. 110 eligible (OPD/ IPD patient with clinical signs and symptoms of Chronic Osteomyelitis & presence of Discharging sinus who gave informed verbal consent) study subjects were selected through consecutive nonprobability sampling. Tubercular Osteomyelitis cases were excluded from study. After thorough sterile cleaning of discharging sinus, 2 pus samples were collected from each patient, first sample was used for gram's staining and ZN staining; second sample was used for inoculation into nutrient agar, blood agar and McConkey agar plates to study staining characteristics, presence of enzymes e.g. oxidase, catalase, coagulase; biochemical reactions, fermentation tests, antibiotic susceptibility testing for by Modified Kirby-Bauer disc diffusion method^[9] (as per the latest Clinical and Laboratory Standards Institute (CLSI) guidelines.^[10]) for Gram positive, Gram Negative bacteria and *Pseudomonas*. Plates were examined after this period for growth of bacterial colonies and characteristics such as colony size, shape, elevation, margins, surface, edges, colour, structure, consistency and emulsifiability. Blood agar was also examined for presence of hemolysis around a colony and any evidence of spreading colonies (swarming). The organism forming colony was further identified by methods specified by Mackie & McCartney. Motility of Gram-Negative Bacilli was tested by Hanging Drop method. Data thus collected were entered into Microsoft excel and analysed in form of tables and figures for appropriate representation.

RESULTS

50% cases had *S. aureus* and 18% had *P. aeruginosa* in their pus samples. Majority of patients were male and almost 1/3rd cases belonged to 16-30 years age group.

S. aureus isolates were observed to be sensitive to Cotrimoxazole & Linezolid. Most of the isolate of *Klebsiella pneumoniae* (83.33%), *E.coli* (88.89%) and all isolates of *Proteus mirabilis* (100%) were sensitive to Meropenem.

All isolates of *Pseudomonas aeruginosa* (100%) and *Acinetobacter baumannii* (100%) were sensitive to Polymyxin B and Colistin.c While all isolates of *Acinetobacter baumannii*(100%) were resistant to Piperacillin, Ceftazidime and Aztreonam.

Table1: Population Characteristics of Chronic Osteomyelitis cases

S. No.	Characteristics	No.	%
1.	Sex		
	Male	81	73.64
	Female	29	26.36
2.	Age Group (years)		
	0-15	12	10.91
	16-30	38	34.54
	31-45	35	31.82
	46-60	14	12.73
	>60	11	10.0
3.	Gram Positive Cocci		
	<i>Staphylococcus aureus</i>	55	50
	CONSCoagulase Negative Staphylococcus Species	3	2.73
4.	Gram Negative Bacilli		
	<i>Pseudomonas aeruginosa</i>	20	18.18
	<i>Klebsiella pneumoniae</i>	12	10.91
	<i>Escherichia coli</i>	9	8.18
	<i>Acinetobacter baumannii</i>	8	7.27
	<i>Proteus mirabilis</i>	3	2.73

Fig1: Isolated Micro-Organisms From Chronic Osteomyelitis Patients

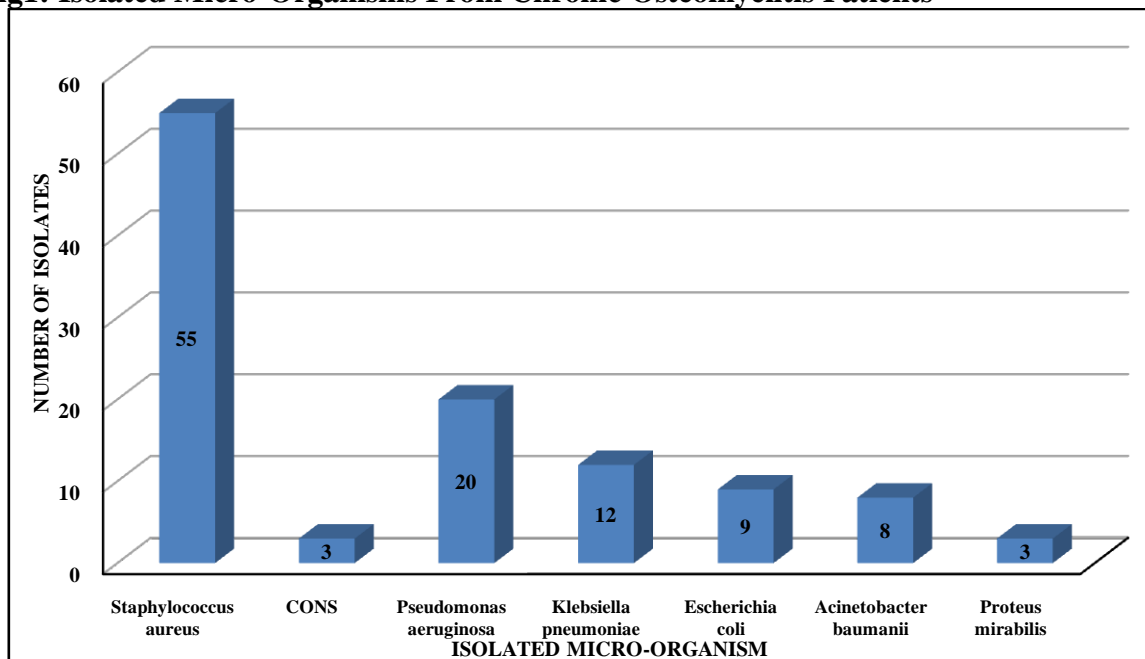
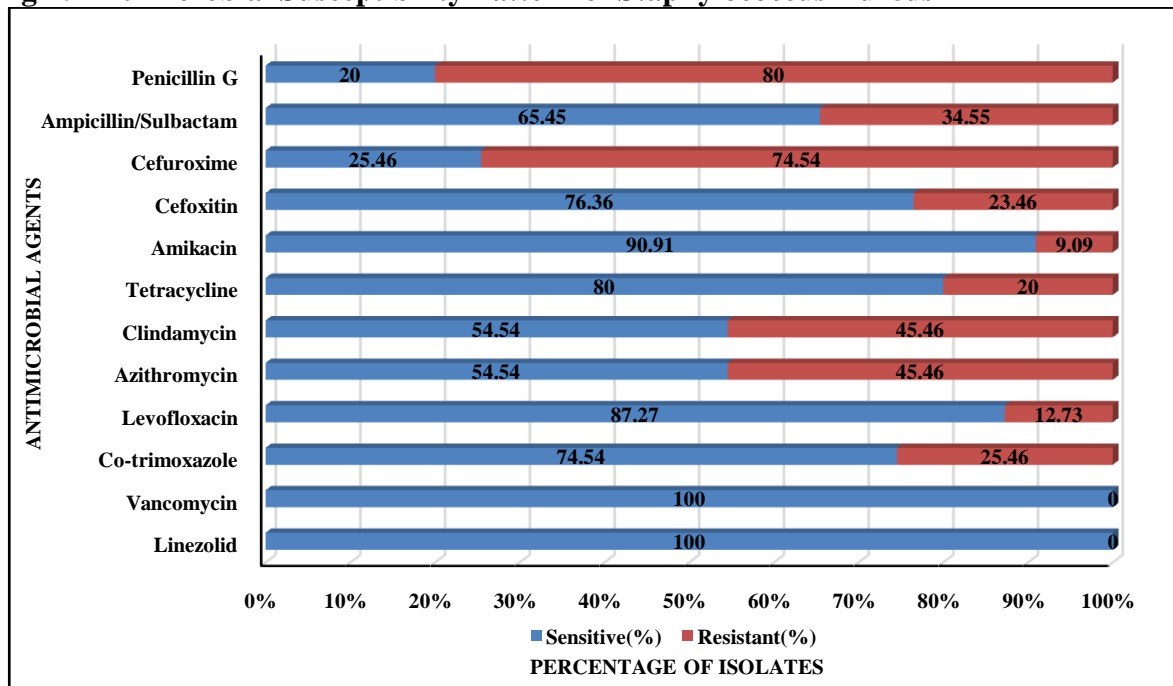


Table2: Antimicrobial susceptibility pattern of Gram-positive isolates

Antimicrobial agent	<i>Staphylococcus aureus</i> (n=55)		CONS (n=3)	
	Sensitive	Resistant	Sensitive	Resistant
Penicillin G (10µg)	11(20%)	44(80%)	0(0%)	3(100%)
Ampicillin/Sulbactam (10µg/10µg)	36(65.45%)	19(34.55%)	2(66.67%)	1(33.33%)
Cefuroxime (30µg)	14 (25.46%)	41 (74.54%)	0 (0%)	3 (100%)
Cefoxitin (30µg)	42 (76.36%)	13 (23.64%)	3 (100%)	0 (0%)
Amikacin (30µg)	50 (90.91%)	05 (09.09%)	3 (100%)	0 (0%)
Tetracycline (30µg)	44 (80%)	11 (20%)	3 (100%)	0 (0%)
Clindamycin (2µg)	30 (54.54%)	25 (45.46%)	0 (0%)	3 (100%)
Azithromycin(15µg)	30 (54.54%)	25 (45.46%)	0 (0%)	3 (100%)
Levofloxacin (5µg)	48 (87.27%)	07 (12.73%)	3 (100%)	0 (0%)
Co-trimoxazole (25µg)	41 (74.54%)	14 (25.46%)	2 (66.67%)	1 (33.33%)
Vancomycin (30µg)	55 (100%)	0 (0%)	3 (100%)	0 (0%)
Linezolid (30µg)	55 (100%)	0 (0%)	3 (100%)	0 (0%)

Fig 2: Antimicrobial Susceptibility Pattern of Staphylococcus Aureus**Table3: Antimicrobial susceptibility pattern of Gram-negative bacilli (fermenters)**

Antimicrobial agent	<i>Klebsiella pneumoniae</i> (n=12)		<i>Escherichia coli</i> (n=09)		<i>Proteus mirabilis</i> (n=03)	
	Sensitive	Resistant	Sensitive	Resistant	Sensitive	Resistant
Ampicillin (10µg)	0(0%)	12(100%)	0(0%)	9(100%)	1(33.33%)	2(66.67%)
Cefotaxime (30µg)	2(16.67%)	10(83.33%)	2(22.22%)	7(77.78%)	1(33.33%)	2(66.67%)
Cefepime (30µg)	7(58.33%)	5(41.67%)	6(66.67%)	3(33.33%)	2(66.67%)	1(33.33%)
Piperacillin +Tazobactam	8(66.67%)	4(33.33%)	5(55.55%)	4(44.45%)	3(100%)	0(0%)

(100+10µg)						
Amikacin (30µg)	7(58.33%)	5(41.67%)	3(33.33%)	6(66.67%)	1(33.33%)	2(66.67%)
Tetracycline (30µg)	6(50%)	6(50%)	2(22.22%)	7(77.78%)	0(0%)	3(100%)
Levofloxacin (5µg)	8(66.67%)	4(33.33%)	8(88.89%)	1(11.11%)	1(33.33%)	2(66.67%)
Co-trimoxazole (25µg)	6(50%)	6(50%)	4(44.45%)	5(55.55%)	0(0%)	3(100%)
Meropenem (10µg)	10(83.33%)	2(16.67%)	8(88.89%)	1(11.11%)	3(100%)	0(0%)

Fig 3: Antimicrobial Susceptibility Pattern Of Klebsiella Pneumoniae

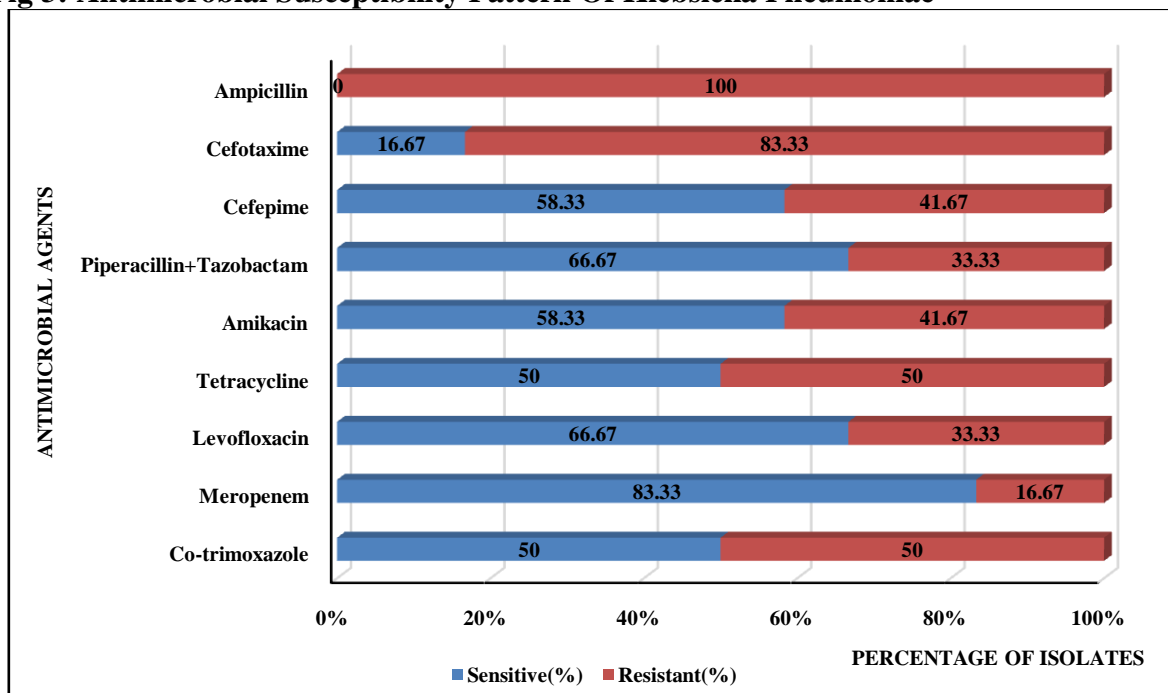
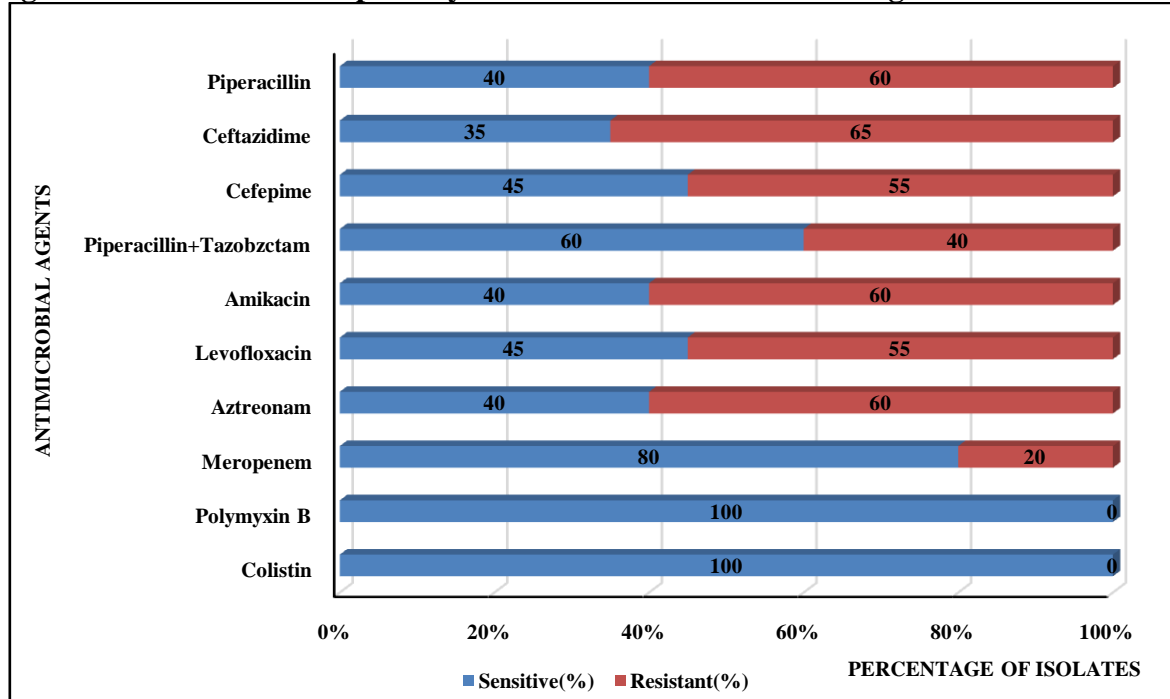


Table4: Antimicrobial susceptibility pattern of Gram-negative bacilli (non-fermenters)

Antimicrobial agent	<i>Pseudomonas aeruginosa</i> (n=20)		<i>Acinetobacter baumannii</i> (n=8)	
	Sensitive	Resistant	Sensitive	Resistant
Piperacillin (30µg)	8(40%)	12(60%)	0(0%)	8(100%)
Ceftazidime(30µg)	7(35%)	13(65%)	0(0%)	8(100%)
Cefepime (30µg)	9(50%)	11(55%)	2(25%)	6(75%)
Piperacillin+Tazobactam(100+10µg)	12(60%)	08(40%)	2(25%)	6(75%)
Amikacin (30µg)	08(40%)	12(60%)	3(37.5%)	5(62.5%)
Levofloxacin (5µg)	09(45%)	11(55%)	5(62.5%)	3(37.5%)
Aztreonam (30µg)	08(40%)	12(60%)	0(0%)	8(100%)
Meropenem (10µg)	16(80%)	04(20%)	3(37.5%)	5(62.5%)
Polymyxin B(300IU)	20(100%)	0(0%)	8(100%)	0(0%)
Colistin(10µg)	20(100%)	0(0%)	8(100%)	0(0%)

Fig 4: Antimicrobial Susceptibility Pattern Of Pseudomonas Aeruginosa

DISCUSSION

Chronic osteomyelitis is one of the most frequent disease and is reported to be a considerable health care burden among the developing countries like India. An increase in the emergence of drug resistant strains makes treatment even more complicated. Hence, area-wise studies on bacteriological profiles and their antimicrobial susceptibility pattern are essential to guide policy on the appropriate use of antibiotics. In our study the incidence of chronic osteomyelitis was high in males (73.64%) and majority of patients were in age groups between 16-45 reason being the younger and middle age groups are more accident prone because of their occupation and lifestyle. These findings correlate with the findings of studies carried out by R. V. Shah et al^[11] with majority of cases i.e. 78% was in age group 20-70 years and S. Chakravarty et al^[12] with majority of cases in age group 16-45 year. *Staphylococcus aureus* was the most commonly isolated micro-organism and this finding correlates with the findings of studies carried out by A. Pandey et al^[14] in which *Staphylococcus aureus* (49.41%) was also most commonly isolated micro-organism. *Pseudomonas Aeruginosa* was the 2nd most commonly isolated micro-organism i.e. 18.18% and this finding correlates with the findings of studies carried out by K. Zerzy et al^[15] in which *Pseudomonas Aeruginosa* (16.12%) was also 2nd most commonly isolated micro-organism. *Klebsiella pneumoniae* was found to be the most commonly isolated micro-organism of *Enterobacteriaceae* family i.e. 10.91% and this finding correlates with the findings of studies by A. Pandey et al^[14] in which *Klebsiella pneumonia* (9.41%) was also most commonly isolated micro-organism of *Enterobacteriaceae* family.

Table-5: Comparison of micro-biological profile with other studies.

Studies	<i>St. aureus</i>	<i>P. Aeruginosa</i>	<i>K. pneumoniae</i>	<i>E. coli</i>	<i>A. baumannii</i>	<i>P. mirabilis</i>	CONS
Ruchi V. Shah ^[11]	60.60%	13.13%	13.13%	4.04%	6.06%	-	3.03%
G. Suguneshwari	53.48%	10.46%	5.82%	-	6.97%	9.30%	13.95%

et al ^[16]							
S. Chakarvarty et al ^[12]	51%	10%	5%	14%	-	7%	4.4%
M.ali et al ^[17]	58%	10%	6%	6%	-	4%	14%
A.pandey et al ^[14]	49.41%	20%	9.41%	17.64%	-	2.35%	20%
K. Zerzy et al ^[15]	38.71%	16.12%	3.22%	12.9%	3.22%	3.22%	9.68%
Dr. Hassani et al ^[18]	42.05%	11.36%	2.26%	1.13%	-	4.54%	4.54%
Present study	50%	18.18%	10.91%	8.18%	7.27%	2.73%	2.73%

Staphylococcus aureus was found to be sensitive to Vancomycin(100%), Linezolid(100%),Amikacin(90.91%) that correlate with the findings of studies by A. Pandey et al ^[14];in that *Staphylococcus aureus* was sensitive to Vancomycin(100%), Linezolid(100%).*Pseudomonas aeruginosa* was sensitive to polymyxin B (100%), Colistin (100%), Piperacillin+Tazobactam (60%).

These findings correlate with the findings in studies by A. pandey et al ^[14] in that *Pseudomonas aeruginosa* was sensitive to Colistin (100%) in which *Pseudomonas aeruginosa* was sensitive toPiperacillin+Tazobactam (75%). micro-organism belonging to *Enterobacteriaceae* familywas found to be sensitive to Meropenem (87.5%), Levofloxacin (70.83%), Piperacillin +Tazobactam(66.66%) that correlate with the findings of G. Suguneshwari et al^[16]in that *Enterobacteriaceae*family was sensitive to Meropenem (92.3%), Levofloxacin (84.6%) and Piperacillin +Tazobactam(84.6%).

Among all the *Staphylococcus aureus* isolates number of strains showing resistance to cefoxitin 30µg which is used as surrogate marker for methicillin resistance (as per CLSI) was found to be in comparable limits with other studies.

Most of the *Staphylococcus aureus* were found to be sensitive to amikacin, vancomycin and linezolid, whereas only three-fourth of the strains were sensitive to cotrimoxazole, tetracycline and levofloxacin. Antibiotic sensitivity pattern of *Pseudomonas aeruginosa*, the most commonly isolated gram-negative bacilli demonstrates resistance to commonly used drugs such as cephalosporins, aminoglycosides and fluoroquinolones, limiting choice of treatment to carbapenems. Another major group of bacteria associated with chronic osteomyelitis is *Enterobacteriaceae* family accounting for total 24 cases.i.e. 21.81% including *Klebsiella pneumoniae*, *Escherichia coli* and *Proteus mirabilis*, of all culture positive cases. This group showed alarming resistance to all drugs under study except for carbapenem group.

CONCLUSION

In all the studies with which present study was compared, though the age sex distribution and bacteriological profile were similar by and large but the sensitivity pattern of anti-bacterial in different groups of organisms isolated varied to large extent highlighting the major demographic variation and need for determining the prevalent anti-biogram of a particular region for proper management of disease.

RECOMMENDATION

Sensitivity of isolates from cases of osteomyelitis differ from place to place and time to time. Therefore, it is important to know the microbiological profile and susceptibility pattern prevalent in particular demographic region to assist clinician in starting a regimen that is most likely to eliminate infection as long as the culture reports confirms the micro-organism and its

sensitivity. Choosing appropriate antibiotics and their judicious use will help in preventing emergence of resistance to the drug which are still sensitive.

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