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Bacteriological Profile in Chronic Osteomyelitis Patientsand TheirAntibiogram in A Tertiary Care Hospital, Jamnagar, Gujarat, India

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Abstract
Introduction: *Chronic Osteomyelitis has been continuing as the most important cause of morbidity among patients with bone infections. Even though early detection of cases and advanced treatments are in process, osteomyelitis is still continued as a major problem due to treatment failures and multidrug resistance. This study was conducted to determine the pus culture of chronic osteomyelitis and their susceptibility pattern to variousantimicrobial drugs.*
Aim: *1)To isolate and identify the Aerobic bacteria causing chronic osteomyelitis in one hundred fifty patients attending Orthopaedic outpatient department and those admitted in Orthopaedic wards of Shri M.P. Shah Government Medical College, Jamnagar. 2)To study the antibiotic sensitivity of the bacteria isolated.* ***Materials and methods:*** *150 pus samples taken aseptically were cultured on Blood and MacConkey agar plates aerobically at 37^{o}C for 18-24 hrs. Culture isolates were identified by a series of standard biochemical reactions. Antimicrobial susceptibility testing was done by Modified Kirby-Bauer's disc diffusion method and the results were interpreted following CLSI guidelines.*
Results: *Study group comprised 108 males and 42 females. Majority of the patients were in the age group of 20 – 70 years 117 (78%) with trauma being the most common 76 (50.67%) predisposing factor. The commonest organisms isolated wereStaphylococcus aureus60 (60.60%) and Pseudomonas aeruginosa13 (13.13%). Majority of Gram positive organisms were sensitive to Linezolid and Vancomycin and Gram negative organisms to piperacillin tazobactam combination and ceftazidime.*
Conclusion: *The wide range of causative organisms and degree of resistance to commonly used anti-microbials supports the importance of pus culture and provides important information to guide clinician's choice of empirical antibiotics.*
Keywords: *Osteomyelitis, S.aureus,P. aeruginosa, antibiotic resistance.*

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I. Introduction

Osteomyelitis is defined as an inflammation of the bone caused by an infecting organism. The infection generally is due to a single organism, but polymicrobial infections can occur, especially in the diabetic foot[1]. Chronic osteomyelitis is a relapsing and persistent infection and is characterized by low-grade inflammation, presence of dead bone (sequestrum), new bone apposition, and fistulous tracts[2]. Chronic osteomyelitis commonly involves long bones; especially tibia and femur[3]. Introduction of microorganisms into the bone may occur during stabilization of the fracture, implanting prosthesis or due to trauma. Microorganisms reach to the metaphysis of bone through blood flow from skin wounds and other infectious regions. Multiplication of microorganisms in metaphysis will cause congestion, oedema, exudates, leucocytosis, necrosis and abscess[4]. The bacteria most commonly causing chronic osteomyelitis are S. aureus, Coagulase negative Staphylococcus,Pseudomonas spp., E. coli, Proteus spp., Klebsiella spp.,Enterococcus spp., Enterobacter spp. and anaerobes likePeptostreptococcus spp., Bacteroides spp., Clostridium spp. and rarely Salmonella spp. and Actinomycetes[5].The still dominant role of Staphylococcusaureus could be confirmed, but also the increasing number of Gram-negative bacteria. Inappropriate and excessive use of antibiotics is considered as the main cause of development of drug resistance. Proper management of chronic osteomyelitis requires accurate microbial isolation and appropriate antibiotic administration. The present study was conducted to study the

bacteriological profile of osteomyelitis along with the antimicrobial susceptibility patterns so as to establish empiric therapy guidelines at the hospital set up**.**

II. Material And Methods

The present study "Bacteriological Study of Chronic Osteomyelitis and their Antibiogram" is a retrospective study conducted in the Department of Microbiology, Shree M.P.Shah Government Medical College, Jamnagar over a period of 1 year from November 2014 to October 2015.

Inclusion Criteria : One hundred fifty consecutive samples of clinically diagnosed patients of chronic osteomyelitis of all agegroups and both sexes attending the Orthopaedic unit of Shree M. P. Shah Government Medical College and Guru Gobind Singh Government Hospital, Jamnagar were studied over a period of 1 year from November 2014 to October 2015.

Exclusion Criteria :1. Patients other than chronic osteomyelitis were excluded from study.
2.Causes other than aerobic bacterial organisms were excluded from study.

Pus samples were collected from depth of the wound under strict aseptic conditions. Direct smear examination was done. The pus sample was inoculated onto blood and MacConkey agar plates and incubated aerobically at 37^{o}C for 18-24 hrs. The isolates were identified by standard procedures[6]. The culture isolates were identified by Gram stain morphology, colony characters and biochemical reactions[7,8]. Antibiotic sensitivity was done on Mueller Hinton agar by Kirby Bauer disc diffusion method as per CLSI guidelines[9]. Antibiotic discs with following concentration were used: Ampicillin (10µg), Amoxicillin clavulinic acid combination(50/10µg), Piperacillintazobactam combination(100/10µg), Gentamicin (10µg), Amikacin (30µg), Ciprofloxacin (5µg),Levofloxacin(5µg),Cefuroxime(30µg), Cefotaxime (30µg), Ceftazidime (30µg), Imipenem (10µg), Erythromycin (15µg), Clindamycin (2µg), Linezolid (30µg), Vancomycin(30µg).

III. Results

The highest incidence of osteomyelitis (78%) was seen in the 20-70 year age group. Majority of patients were males 108(72%) than females 42 (28%).The commonest bone affected was femur 65(43.33%) with trauma being the commonest predisposing factor 46(50.67%). Other predisposing factors include post-operative infections, orthopaedic implants and immunocompromised. Out of 150 samples, 96(64%) was culture positive and 54(36%) culture negative, monomicrobial flora was seen in 93(62%) and polymicrobial flora 3(2%).Isolated Gram positive bacteria wereStaphylococcus aureus60(60.60%), Enterococcus spp 3(3.03%) and Gram negative bacteria were Psedomonasaeruginosa 13(13.13%), Klebsiellaspp 13(13.13%), Acenetobacterspp 6(6.06%) and Escherichia coli 4(4.04%). Most of the Gram positive isolates were sensitive to Vancomycin and Linezolid and Gram negative isolates to Piperacillintazobactam combination and ceftazidime.

Table 1: Showing culture reports of chronic osteomyelitis

Culture report	No. of swabs studied	Percentage(%)
Culture positive	96	64
Culture negative	54	36
Total	150	100

Table 2: Various organisms isolated

Organisms	No. of organisms	Percentage(%)
Staphylococcus aureus	60	60.60
Psedomonasaeruginosa	13	13.13
Klebsiellaspp	13	13.13
Acinatobacterspp	06	6.06
Escherichia coli	04	4.04
Enterococcus spp	03	3.03
Total	99	100

Table 3: Predisposing factors for chronic osteomyelitis

Predisposing factor	No. of cases	Prcentage(%)
Postoperative	40	26.67
Trauma	76	50.67
Orthopaedic implants	21	14
Immunocompromise	9	6
Idiopathic	4	2.67

Table 4: Involvement of various bones

Bones involved	No. of cases	Percentage(%)
Femur	65	43.33
Tibia	40	26.67
Radius	12	8
Humerus	10	6.67
Fibula	6	4
Patella	6	4
Ulna	5	3.33
Metacarpals	3	2
Metatarsals	2	1.33
Calcanuem	1	0.67

Table 5: Antibiotic sensitivity pattern of Gram positive organisms

Organisms	No. of Isolates	Antibiotics								
		AMP	AMC	AN	L	CD	CXM	CTX	CIP	E
S. aureus	60	3	7	50	59	50	20	24	23	41
		(5%)	(11.67%)	(83.33%)	(99.33%)	(83.33%)	(33.33%)	(40%)	(38.33%)	(68.33%)
Enterococcus spp.	03	1	1	3	3	1	1	1	2	2
		(33.33%)	(33.33%)	(100%)	(100%)	(33.33%)	(33.33%)	(33.33%)	(66.67%)	(66.67%)

(AMP = ampicillin; AMC = amoxy-clavulanic acid; AN = vancomycin; L=linezolid; CD = clindamycin; CXM = cefuroxime; CTX = cefotaxime; CIP = ciprofloxacin; E = erythromycin)

Table 6: Antibiotic sensitivity pattern of Gram negative organisms

Organisms	No. of Isolates	Antibiotics						
		AK	CTX	CAZ	IPM	LE	CIP	GE
Pseudomonas aeruginosa	13	7	7	12	5	4	2	2
		53.84%	53.84)	92.30%	38.43%	30.76%	15.38%	15.38
Klebsiella pneumonia	13	4	5	6	4	4	4	3
		30.76%	38.43%	46.15%	30.76%	30.76%	30.76%	23.07
Acinatobacterspp	06	1	-	-	2	2	-	-
		16.67%	-	-	33.33%	33.33%	-	-
Escherichia coli	04	3	-	-	1	2	2	3
		75%	-	-	25%	50%	50%	75%

(AK = amikacin; CTX = cefotaxime; CAZ = ceftazidime; IPM = imepenem; LE = levofloxacin ; CIP=ciprofloxacin; GEN = gentamicin; PT = piperacillin – tazobactam)

Discussion

Osteomyelitis is one of the most inconvenient diseases among most of the developing countries like India. An increase in the emergence of drug resistant strains makes treatment even more complicated.Chronic osteomyelitis may require antimicrobial therapy for months to years. Widespread use of antibiotics has altered aetiological pattern of infections and antibiotic susceptibility. Hence continuous monitoring of susceptibility pattern needs to be carried out in individual setting so as to detect the true burden of antibiotic resistance among organisms and prevent their further emergence by judicious use of drugs[10]. In the present study chronic osteomyelitis was commonly seen in males of 20- 70 years age group with trauma being the commonest cause which leads to epiphysial cell destruction and hemorrhage which in turn decreases tissue resistance **[11].** Other causes included post-operative infections, orthopaedic implants and immunocompromised.There were 64% culture positive and 36% culture negative samples. Collection of specimen before the administration of antibiotics and use of proper transport media play a role in reducing the incidence of false negative cultures[12].

Monomicrobial flora was seen in 62%, polymicrobial flora 2%and the commonest bone affectedwas femur 43.33% which is consistent with the studies by Zuluaga AF et al[2].and Perry CR et al[13].Staphylococcus aureusfollowed by Pseudomonas aeruginosawas common organisms isolated which is similar to study by Patzakis MJ et al[14]and in study by Saurabh A et al[15].Similar study of Wadekar et al showed 87% culture positive and 13% culture negative, 67% monomicrobial and 20% polymicrobial, 32.9% Staphylococcus aureus and 15.8% Pseudomonas aeruginosa[16]. Other similar study of Vishwajith et alshowed 94.88% culture positive 5.12% culture negative, 73.46% monomicronial and 21.42% polymicrobial, 48.69% Staphylococcus aureus, 13.04% Pseudomonas aeruginosa[17]. In present study most of the Gram positive organisms were sensitive to vancomycin and linezolid which is similar to study by Wadekar at al and Vishwajith et al[16,17]. In the present study Gram negative organisms were sensitive topiperacillintazobactam combination and ceftazidime which is similar to findings in study byWadekar at al that showed Gram negative organisms were sensitive to imipenam and amikacin and in study by Vishwajith et al showed that Gram negative organisms were sensitive to piperacillintazobactam combination, imipenam and amikacin[16,17].

IV. Conclusion

Chronic Osteomyelitis has been the major cause of morbidity since long. Emerging multidrug resistant strains is of major concern to treat the disease. Even though gram negative bacteria are being increased significantly but still Staphylococcus aureus is being continued as a major etiological agent of chronic osteomyelitis.Chronic osteomyelitis is the common form of osteomyelitis in adults and is usually the sequel of trauma. Our study thereby will guide the clinician in choosing appropriate antibiotics which not only contribute to better treatment but there judicious use will also help in preventing emergence of resistance to the drug which are still sensitive .

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