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ORIGINAL ARTICLE

BACTERIAL FLORA IN DIABETIC ULCER

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ABSTRACT: BACKGROUND: Diabetic foot infections are one of the most feared complications of diabetes. This study was undertaken to determine the common etiological agents of diabetic foot infections and their in vitro antibiotic susceptibility. **METHODS:** A prospective study was performed over a period of two years in a tertiary care hospital. The aerobic and anaerobic bacterial agents were isolated and their antibiotic susceptibility pattern was determined. **RESULTS:** One hundred patients with Diabetic ulcer were studied, of which 65 were males and 35 were females. Majority of patients were in the age group of 51 to 60 years (37%) and polymicrobial etiology was 64 % and monomicrobial etiology was 36%. A total of 187 organisms were isolated of which 165 were aerobic and 22 were anaerobic. Most frequently isolated aerobic organisms were Pseudomonas Sp., Klebsiella Sp., E coli Sp., and Staphylococcus aureus. The common anaerobic organisms isolated were Peptostreptococcus Sp. And Bacterioids Sp. **CONCLUSION:** High prevalence of multi-drug resistant pathogens was observed. Amikacin, Imipenem were active against gram-negative bacilli, while vancomycin was found to be active against gram-positive bacteria.

KEYWORDS: Diabetic Ulcer, Microbial Flora, Imipenam.

INTRODUCTION: Diabetes mellitus is a chronic disorder and affects large segments of population.[1] The world wide prevalence of diabetes now exceeds 200 million and is predicted to rise to more than 300 million in the next 20 years.[2] Diabetes mellitus is the major cause of long term damage, dysfunction and failure of various organs, especially the eyes, kidneys, nerves, heart and blood vessels.[3] Diabetic foot tends to be under recognized as a health issue, despite the fact that ulceration, gangrene and amputation are common complications of diabetes. Diabetic foot ulcer affects 10-15% of patients with diabetes during their life time. Infected, non-healing ulcer is the major cause of non-traumatic lower limb amputation. It is estimated to be 40 times greater than those in the non-diabetics. Over 1 million amputations for diabetes related complications occur every year.[2] The reason for the increased incidence of diabetic ulcer involve the interaction of several pathogenic factors like neuropathy, abnormal foot biomechanics and peripheral arterial disease.[4] Infection in lower extremity follows a traumatic injury or breakthrough of the skin with introduction of bacteria. The most important characteristic of the diabetic ulcer infection is that, it is commonly polymicrobial in nature. In superficial wounds, aerobic bacteria are predominant pathogens. Anaerobic organisms are found more frequently in deeper wounds. Staphylococcus spp., Streptococcus spp., Enterococcus spp., species of Enterobacteriaceae and Pseudomonas spp. are the most common aerobic isolates. Peptostreptococcus spp. and Bacteroides spp. are the most common anaerobic isolates.[5] Frequency of fungal isolations from the diabetic foot ulcer differ significantly, Candida spp. is the most commonly isolated yeast from these ulcers [6]

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Proper management of these infections requires microbial isolation and appropriate antibiotic selection. The present study was undertaken to determine the microbial flora of diabetic ulcer using optimal culture techniques and the antimicrobial sensitivity pattern of the isolates.

METHODOLOGY: The present study "Bacterial Flora in Diabetic Ulcer" was conducted in the Department of Microbiology, Kurnool Medical College Kurnool, from Aug 2011 to July 2013, total of one hundred patients with diabetic ulcer admitted in surgical and Endocrinology wards of Government General Hospital, Kurnool were studied. Also 20 patients of Non diabetic ulcer were taken as controls.

Inclusion Criteria: One hundred patients with diabetic ulcer of Wagner's grade I and above were included.

History taking and examination: A proforma was filled for each patient documenting, age, sex, address and clinical information including chief complaints, duration of symptoms, predisposing factor and any previous history of treatment.

Collection of sample: The surface of the ulcer was rinsed with sterile normal saline, superficial exudate was debrided using a sterile instrument. [7] Non-involved adjacent skin was sterilized with iodine and 70% alcohol.[8,9]

Three swabs were collected from each patient. The sterile, cotton tipped swabs were moistened with sterile saline before collecting the specimen. One swab was used for the isolation of aerobic bacteria. Second swab was collected and transported in Thioglycollate broth and processed in anaerobic jar for the isolation of anaerobic organisms. The third swab was used for preparation of smear for gram stain.[10]

Debrided necrotic material was also collected.

After sample collection, the specimens were processed immediately in the laboratory.

For Aerobic organisms, the swab was inoculated on nutrient agar, blood agar and MacConkey agar. All plates were incubated aerobically at 37°C and evaluated at 24 hours, 48 hours and 72 hours. The organisms isolated were identified using standard techniques, based on the colony morphology, Gram staining of smear from colony and biochemical properties.

For Anaerobic organisms, the swab was first inoculated on neomycin blood agar and then transferred to thioglycollate medium. After inoculation of the sample on neomycin blood agar, a metronidazole disc was placed at the inoculation site. The inoculated media were immediately placed in McIntosh Fildes anaerobic jar and incubated at 37°C for 48 hours. Anaerobiasis was achieved.

RESULTS: One hundred patients with diabetic ulcer and 20 patients of non-diabetic ulcers admitted in the surgical wards and Endocrinology Department of Government General Hospital, Kurnool were studied. In non-diabetic ulcers Monomicrobial flora were observed while in diabetic ulcers, polymicrobial flora were observed.

The clinico-microbiological analysis from the 100 diabetic foot ulcer patients studied, were as follows:

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AGE GROUP	MALES	FEMALES
	No.	No.
21-30	2	0
31-40	1	2
41-50	14	13
51-60	27	10
61 and Above	21	10
TOTAL	**65**	**35**

Table 1: Age and Sex distribution of 100 cases of Diabetes Mellitus

The above table shows that most of the patients with diabetic ulcer were above 50 years of age.

Duration of diabetes mellitus in years	Number of cases	Percentage
< 1 yr	4	4
1-5 yrs	39	39
6-10 yrs	44	44
11-15 yrs	13	13
Total	**100**	**100**

Table 2: Duration of Diabetes mellitus

The above table shows that 4 cases were detected within one year prior to the time of admission for the treatment of ulcer.

Type of diabetes mellitus	Number of cases	Percentage
IDDM	1	1
NIDDM	99	99

Table 3: Type of Diabetes mellitus

IDDM: Insulin dependent diabetes mellitus; NIDDM: Non-insulin dependent diabetes mellitus.

DURATION IN WEEKS	No. OF CASES	PERCENTAGE
<1 WEEK	3	3
2-4 WEEKS	39	39
5-7 WEEKS	16	16
8-10 WEEKS	30	30
>11 WEEKS	12	12
TOTAL	**100**	**100**

Table 4: The duration of Diabetic ulcer

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The above table shows that most of the patients presented with ulcer for more than two weeks duration without any difference in the floral composition in new or old ulcers. Both were polymicrobial.

TYPE OF ORGANISM	No. OF CASES	PERCENTAGE
AEROBIC	79	79
ANAEROBIC	0	0
AEROBIC AND ANAEROBIC	21	21
NO GROWTH	0	0
TOTAL	**100**	**100**

Table 5: Organisms isolated from the Diabetic ulcer

The above table clearly indicates that no pure anaerobes were isolated.

Type of organisms	Number of organisms	Percentage
Gram positive organisms	37	22.4
Staphylococcus aureus	24	14.5
Staphylococcus epidermidis	4	2.4
Enterococcus faecalis	9	5.5
Gram negative organisms	128	77.6
Pseudomonas aeruginosa Non-pigment producing pseudomonas	36	21.9
Klebsiella pneumoniae Klebsiella oxytoca(2)	32	19.4
E. coli	26	15.8
Proteus mirabilis Proteus vulgaris(2)	23	13.9
Providencia rettgeri	7	4.2
Citrobacter freundii(3) Citrobacter koseri(1)	4	2.4
Total	**165**	**100**

Table 6: Different aerobic organisms isolated in Diabetic ulcer cases

Type of organisms	Number of organisms	Percentage
Peptostreptococcus spp.	10	45.5
Bacteroides fragilis	7	31.8
Prevotella spp.	3	13.6
Porphyromonas spp.	2	9.1
Total	**22**	**100**

Table 7: Different anaerobic organisms isolated from Diabetic ulcers

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The above table shows that there was no apparent relation between the age of the ulcer and the anaerobes isolated.

Type of organisms	Number of organisms	Percentage
Pseudomonas spp. + Peptostreptococcus spp.	4	19.05
Proteus spp. + Pseudomonas spp. + Bacteroides spp.	3	14.29
E. coli + Pseudomonas spp. + Peptostreptococcus spp.	3	14.29
Klebsiella spp. + Pseudomonas spp. + Bacteroides spp.	2	9.53
S. aureus + Bacteroides spp.	1	4.76
E. coli + Proteus spp. + Peptostreptococcus spp.	1	4.76
Enterococcus spp. + E. coli + Porphyromonas spp.	1	4.76
E. coli + Peptostreptococcus spp. + Prevotella spp.	1	4.76
S. aureus + Proteus spp. + Prevotella spp.	1	4.76
S. aureus + Pseudomonas spp. + Porphyromonas spp.	1	4.76
Klebsiella spp. + Proteus spp. + Peptostreptococcus spp.	1	4.76
Klebsiella spp. + E. coli + Peptostreptococcus spp.	1	4.76
S. aureus + Prevotella spp.	1	4.76
Total	**21**	**100**

Table 8: Distribution of aerobic and anaerobic organisms in polymicrobial flora

The above table shows the presence of aerobic and anaerobic organisms isolated in polymicrobial flora.

	Enterobacteriaceae					Non fermentors
	E.coli n=26	**Proteus n=23**	**Klebsiella n=32**	**Citrobacter n=4**	**Providencia n=7**	**Pseudomonas n=36**
Amikacin	0	0	20	0	0	15
Ampicillin	100	100	100	100	100	-
Aztreonam	45.4	40	80	60	100	53
Carbenicillin	-	-	-	-	-	8
Cefazolin	82	100	100	100	100	-
Cefuroxime	73	80	60	100	0	-
Cefotaxime	73	80	60	100	0	-
Ceftazidime	-	-	-	-	-	61
Cefepime	45.5	40	40	75	0	46
Cefaperazone Sulbactam	18.1	20	20	25	0	0
Ciprofloxacin	54.5	80	80	75	0	46
Chloramphenicol	0	0	40	100	0	-
Colistin	0	100	0	0	0	0

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Cotrimoxazole	82	100	80	100	0	-
Gentamicin	9	100	100	100	0	61.5
Imipenem	0	0	0	25	0	0
Meropenem	45.4	40	20	75	0	23
Ofloxacin	54.5	80	80	75	0	53.4
Piperacillin Tazobactam	27.2	0	20	100	0	23
Polymixin B	0	100	0	0	0	0
Tetracycline	54.5	0	60	0	0	-
Tobramycin	55	40	100	50	0	38.4

Table 9: Antibiogram: Antibiotic Resistance pattern of Gram Negative Bacilli (% of Resistance)

	Staphylococcus aureus n=24	**CoNS n=4**	**Enterococci n=9**
Penicillin	100	100	37.5
Ampicillin	-	-	50
Cefazolin	100	100	62.5
Cloxacillin	100	-	-
Ciprofloxacin	50	50	-
Ofloxacin	50	50	0
Gentamicin	60	50	-
Netilmicin	10	0	-
Cotrimoxazole	40	-	-
Tetracycline	80	-	-
Erythromycin	40	50	50
Clindamycin	20	50	25
Chloramphenicol	20	50	37.5
Vancomycin	0	0	0
Teicoplanin	0	0	0
Linezolid	0	0	0

Table 10: Antibiotic Resistance pattern of the Gram positive cocci.(% of resistance)

DISCUSSION AND CONCLUSION: Diabetic ulcer is a multifaceted problem, primarily due to the underlying neuropathy, ischemia and infection. Each of these factors acting alone or in concert, predisposes to ulceration when subjected to mechanical, thermal and chemical trauma. [11] Infection usually follows ulceration or injury to the neuropathic or ischemic foot. Superimposed infection constitutes a medical emergency threatening both the limb and life. Infection is usually of polymicrobial etiology. A superficial infection is usually caused by aerobic bacteria and deep infection is caused by anaerobes.[12] Foot problems in diabetes can produce not only a physical disability but is also a socio-economic problems.[13] In our study, most of the gram negative organisms were

susceptible to Amikacin and Imipenem while most of the gram positive organisms were susceptible to Vancomycin. All the anaerobic organisms were susceptible to Metronidazole.

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