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RESEARCH ARTICLE

THE STUDY OF BACTERIOLOGICAL PROFILE OF DIABETIC FOOT ULCER WITH A SPECIAL REFERENCE TO ANTIBIOGRAM IN AND AROUND PATNA, BIHAR

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ABSTRACT

Introduction: A diabetic foot infection is one of the leading causes of the hospitalisation among diabetic patients. Many studies have reported on the bacteriology of diabetic foot infections over the past 25 years but the results have been varied. Diabetic foot wounds are commonly infected, and hence infection leads to the formation of microthrombi causing further ischaemia, necrosis and progressive gangrene. **Material and methods:** Samples were collected from 90 cases of diabetic foot ulcer by using sterile swabs and they were processed as per the clinical and laboratory standard Institute guidelines. **Results:** Out of 90 cases of diabetic foot ulcer 50 cases (62.5%) were monomicrobial and 30 cases (37.5%) were polymicrobial. In our study gram negative bacilli were isolated in preponderance than gram positive cocci. Among gram negative bacilli the commonest isolate was *pseudomonas* spp (36%), followed by *E. coli* (28%) and *Staphylococcus aureus* (20%). The antibiotic sensitivity pattern of the bacteria were also studied. **Conclusion:** This study showed raised incidence of multidrug resistance isolates from the diabetic foot ulcer cases. Hence knowledge of antibiotic sensitivity pattern is essential for determining appropriate treatment of diabetic foot ulcer.

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INTRODUCTION

A diabetic foot ulcer is a frequent and the most serious complication of diabetes mellitus. Diabetic ulcers are most common foot injuries leading to lower extremity amputation. It is characterised by several pathological complications such as neuropathy, Peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, which leads to formation of microthrombi causing further ischaemia, necrosis, development of gangrene and necessitates limb amputation. Diabetes mellitus is one such metabolic disorder that impedes the normal steps of wound healing process. Many studies show a prolonged inflammatory phase in diabetic wounds, which causes a delay in the formation of mature granulation tissue and a parallel reduction in wound tensile strength (McLennan, 2006). Impaired microvascular circulation limits the access of phagocytic cells to infected area and this results in poor concentration of antibiotics in infected tissue (Bronze, 2016). Between 10 and 15 percent of those with diabetes can expect to develop a foot ulcer at some point in their lives (Millians, 2008). The Indian diabetic population is expected to increase to 57 million by the year 2025 (Shakil, 2010). These infections are polymicrobial in nature. *E. coli*, *Proteus* spp, *Pseudomonas* spp, *Staphylococcus aureus* and *Enterococci* spp are reported as frequent organisms isolated from cases of diabetic foot ulcer (Gadepalli, 2008).

The diversity of bacterial population in chronic wounds are an important cause for the chronicity of the diabetic foot ulcer and patients usually require several episodes of hospitalization. Exposure of several antibiotics increase the risk of developing Multi drug resistant infections. The presence of MRSA and ESBL strains further worsen the prognosis and increase the risk of amputation (Juan Gonzalez Del Castillo, 2012). So proper management of diabetic foot ulcer requires selection of appropriate antibiotics based on the culture and the antimicrobial testing. The present study was conducted to evaluate the different microbes infecting diabetic foot ulcer and to know their antibiotic sensitivity pattern.

MATERIAL AND METHODS

This study was carried out in Department of microbiology Patna medical College, Patna. 90 cases selected for this study were known diabetic patients with foot ulcers attending IPD and OPD. A detailed history comprising of the name, age, sex, address, occupation, duration of diabetes, the type of treatment for diabetes received earlier and the presence of other systemic illnesses. The patients were also assessed clinically and the ulcers were graded according to Wagner's grade. After obtaining informed consent from the patient, samples (purulent discharge or curetted material) were collected by using two sterile swabs. Samples were collected by making a firm, rotatory movement with the swabs. One swab was used for

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Gram's staining and the other was used for culture. The specimens were inoculated onto blood agar, chocolate agar, Macconkey's Agar and thioglycollate medium. The organisms were identified on the basis of their colony morphology, gram's staining properties and their biochemical reactions.

Antibiotic susceptibility testing

The AST was done by the Modified Kirby Bauer disc diffusion method as per the CLSI guideline, 2018.

RESULTS

Bacteria isolated from diabetic foot ulcers

Bacteria	No. of isolates (Mono microbial)	Percentage	No. of isolates (Poly microbial)
<i>Staphylococcus aureus</i>	10	20	12
<i>Enterococcus spp.</i>	2	4	2
<i>Escherichia coli</i>	14	28	16
<i>Pseudomonas aeruginosa</i>	18	36	16
<i>Acinetobacter spp.</i>	1	2	0
<i>Proteus spp.</i>	2	4	2
<i>Klebsiella spp.</i>	3	6	12

Antibiotic Resistance Pattern of Gram Positive Cocci (% of Resistance)

Name of organisms	Antibiotics (% of Resistance)								
	E	C	AMP	CIP	T	CD	OX	V	LZ
<i>Staphylococcus aureus</i>	22	60	20	26	40	40	20	-	-
<i>Enterococcus spp.</i>	30	-	10	25	18	50	-	-	-

E- Erythromycin; C- Chloramphenicol; AMP- Ampicillin; CIP- Ciprofloxacin; T- Tetracycline; CD- Clindamycin; OX- Oxacillin; V- Vancomycin; LZ- Linezolid

Antibiotic Resistance Pattern of Gram Negative Bacilli (% of Resistance)

	<i>Pseudomonas aeruginosa</i>	<i>Escherichia coli</i>	<i>Klebsiella spp.</i>	<i>Proteus spp.</i>	<i>Acinetobacterspp.</i>
AK	14	-	45	72	30
AMC	65	20	88	81	86
AZ	50	40	80	46	48
CFT	5	67	80	48	-
CTZ	10	-	72	30	80
CPM	5	53	68	40	60
CFS	-	-	-	25	-
CIP	46	58	78	68	46
MRP	10	32	10	24	-
PTZ	-	22	-	-	12

AK- Amikacin; AMC- Amoxycylav; AZ- Azithromycin; CFT- Cefotaxime; CTZ- Ceftazidime ; CPM- Cefepime; CFS- Cefoperazone/ Sulbactam; CIP- Ciprofloxacin; MRP- Meropenem; PTZ- Piperacillin/ Tazobactam

Out of 90 cases of DFU bacteria were isolated from 80 cases. 10 cases were found sterile. Out of 80 cases, 50 cases (62.5%) were monomicrobial and 30 cases (37.5%) were polymicrobial. In this study gram-negative bacilli were isolated more frequently than gram positive cocci. The commonest isolate was *Pseudomonas spp* (36%), followed by *Escherichia coli* (28 %), followed by *Staphylococcus spp*(20%). Other isolates were *Klebsiella spp* (6%), *proteus*(4%) and *Enterococcus* (4%) and *Acinetobacter* (2%). In this study, among gram positive organisms *Staphylococcus aureus* were 100% sensitive for Vancomycin and Linezolid. *Enterococcus* were also 100% sensitive for Vancomycin and Linezolid. As for gram negative bacilli, *Pseudomonas* showed 40% resistance to 3rd generation and 35% to 4th generation cephalosporin and 10% resistance to meropenem. It was 100% sensitive to Cefoperazone-Sulbactam and piperacillin-tazobactam. With regard to *E. coli* 67% resistance was shown to Cefotaxime, followed by 58% resistance to ciprofloxacin, 53% resistance to cefepime, 40 % resistance to azithromycin, 32 %resistance to meropenem and 22% resistance to

piperacillin tazobactam. Amikacin, Ceftazidime and Cefoperazone sulbactam were 100% sensitive to *E. coli*. Regarding resistance pattern shown by *Klebsiella* 88% resistance was shown to amoxicillin clavulanic acid, 80% resistance was shown to both azithromycin and cefotaxime, 78% resistance to ciprofloxacin, 68% resistance to cefepime, 45% resistance to amikacin and 10 % resistance to meropenem. Cefoperazone sulbactam and piperacillin tazobactam were 100% sensitive to *Klebsiella*. Regarding resistance pattern of *Proteus spp*, 72% resistance was shown to

amikacin, 68% resistance to ciprofloxacin, 48% resistance to cefotaxime, 46% resistance to azithromycin, 42% resistance to meropenem, 40% resistance to cefepime, 30% resistance to Ceftazidime. Piperacillin tazobactam showed 100% sensitivity to *Proteus*. Regarding sensitivity to *Acinetobacter*, it showed 100% sensitivity to cefotaxime, cefoperazone sulbactam and meropenem.

DISCUSSION

In this study single bacteria isolate was seen in 62.5% and mixed bacterial growth was seen in 37.5%. Mohd Zubair *et al.* and Pappu K *et al.* reported 56.6% and 92% monomicrobial infections and 33% and 7.7% polymicrobial infections respectively which is similar to my study (Zubair, 2010 and Pappum, 2012). Microbiological evaluation of diabetic foot ulcer showed preponderance of gram negative organisms over gram positive organism. In the study of Benwan *et al.*, which was done in Kuwait, they reported that more gram-negative pathogens (51.2%) were isolated than gram positive pathogens

(32.3%) or anaerobes (15.3%) (Zubair, 2010; Al Benwan, 2012 and Anderson, 2005). Among gram-negative bacilli commonest isolate was pseudomonas spp (36%) followed by E. coli (28%) then Klebsiella spp which concur with previous studies. Furthermore, Staphylococcus aureus and Enterococcus culture isolates showed 100% sensitivity to Vancomycin and Linezolid. A study by Al Benwan *et al.* showed same result. Most of gram negative bacterial culture isolates showed 100% sensitivity to cefoperazone sulbactam and piperacillin tazobactam combination.

Conclusion

Both gram positive cocci and gram negative bacilli caused diabetic foot infections with preponderance of gram negative bacilli. Monomicrobial infection was more common than polymicrobial infection in case of diabetic foot infections. Presence of multidrug resistant organisms was high in diabetic foot infections. So appropriate usage of antibiotics based on antibiogram pattern can certainly help the clinician in reducing the burden of diabetic foot ulcer.

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