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Clinical and bacteriological profile of diabetic foot infections in Pattukkottai area hospitals, Tamilnadu, India

C.Manikandan* and P. Prabhakaran

Department of Microbiology, Marudupandiyar College of Arts and Science, Thanjavur, Tamilnadu, India

Corresponding author

KEYWORDS

Diabetes mellitus, Foot ulcer, Infection, Culture sensitivity pattern, Multi-drug resistance

A B S T R A C T

A diabetic foot ulcer is one of the leading causes of the hospitalization among diabetic patients. This study was conducted to know the clinical and sensitivity profile of diabetic foot ulcer patients. Total 134 pus samples were collected from patients having diabetic foot ulcer. Samples were processed as per standard guidelines. All the pus samples yielded growth of organisms making total of 148 isolates. Out of 148 bacterial isolates, 85 were gram negative bacilli and 58 were gram positive cocci. In gram positive organism *Staphylococcus aureus* (17%) was the predominant isolate followed by *CONS* (12%), while *Escherichia coli* (20%) was the predominant isolate followed by *Pseudomonas* spp., (18%) in gram negative organism. The Gram negative bacteria showed good activity against amikacin, cefotaxime, ceftazidime, ceftriaxone, gentamicin and imipenem. *E. coli*, *Klebsiella* spp., and *Proteus* spp., showed 55.5% to 100% resistance to amoxyclav and ampicillin. *Pseudomonas* spp., showed 100% resistance to ampicillin and 96.2% to amoxyclav. All the Gram-positive bacteria showed good sensitivity to most of the antibiotics. It showed good sensitivity for amikacin, gentamycin, amoxyclav, ofloxacin, ciprofloxacin and vancomycin. *E. coli*, *Pseudomonas* spp., and *Staphylococcus aureus* were the most common cause of infections. Most isolates were multi-drug resistance.

Introduction

Diabetes Mellitus (DM) is a serious public health problem worldwide. About 150–170 million populations are suffering from this diseases worldwide and the prevalence of diabetes will be double by 2025 as per WHO reports (Wild *et al.*, 2004). According to world health organization (WHO) the top 10 countries with high number of diabetics are India, China, USA, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh. The estimates for India include 31.7 million in the year 2000 to a drastic increase to 79.4 million diabetics by the year 2030 (Zimmet *et al.*, 2001). Foot ulcer is frequent complication of patients suffering with diabetes mellitus (DM),

accounting for up to 20% of diabetes-related hospital admission (Al-salihi and Israa, 2013).

This wound infection begins superficially, but with delay in treatment and impaired body defense mechanisms, can spread to the other subcutaneous tissues and to deeper structures ultimately leading to dreaded complications such as gangrene and amputations (Hefni *et al.,* 2013). Poorly controlled diabetes is prone to skin infections because elevated blood sugar reduces the effectiveness of bacteria fighting cells. Even small cut may progress to a deep, open sore, called an ulcer (Hena and Lali, 2011). The ulcers become infected, and can develop in the skin, muscle or bone of the foot as a result of the nerve damage and poor circulation as a major causal factor for lower limp amputation (Al-Salihi and Israa, 2013). These infections are polymicrobial in nature. *Escherichia coli*, *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus* and *Enterococcus* spp., are reported as frequent organism isolated from cases of diabetic foot infections (Gadepalli *et al.*, 2006). The aim of present study is to investigate the clinical, causative pathogens and sensitivity profile of diabetic foot ulcer patients.

Material and Methods

This prospective study was done on 134 diabetic patients, previously diagnosed or newly diagnosed as diabetics and presenting with lower extremity infection and were attending the various hospitals in Pattukkottai area, Tamilnadu, India. The study was conducted over a period of one year. Patients included were briefed about the study and details regarding age, sex, type of diabetes, duration of diabetes mellitus and duration of foot infection were recorded. Various specimens (pus, wound exudates or tissue biopsy) for microbiological study were obtained from ulcer region. Surface of the ulcer region was rinsed with sterile normal saline and the pus was collected with sterile cotton swab. The specimens were cultured on Nutrient agar, MacConkey agar, Blood agar and UTIchrom agar. The plates were then aerobically incubated at 37°C for overnight. All the bacteria were isolated and identified using morphological, microscopy and biochemical tests following standard procedures described by Sharma (2008).

Antibiotic sensitivity test was carried out by disc diffusion technique on Muller Hinton agar plates (Bauer *et al.,* 1966). The following antibiotics such as ampicillin (10 mcg), amikacin (30 mcg), gentamicin (10 mcg), ofloxacin (5 mcg), ciprofloxacin (5 mcg), ceftazidime (30 mcg), imipenem (10 mcg), cefotaxime (30 mcg), ceftriaxone (10 mcg), methicillin (5 mcg), erythromycin (15 mcg), clindamycin (2 mcg), vancomycin (30 mcg) and amoxicillin/clavulanic acid (20/10 mcg) were used to determine antibiotic susceptibility pattern. Isolated colonies were picked up from a fresh isolation plate, inoculated on Trypticase Soya broth medium and incubated for 2 to 6 hrs at 37°C until good visible growth. A lawn of test pathogen was prepared by evenly spreading with the surface of the agar plate. The plates were allowed to dry before applying antibiotic disc. The antimicrobial discs were placed at equal distance and the discs were pressed gently with forceps. After 16–18 hrs incubation of the plates at 37°C, the zone of inhibition were read with metallic rulers in mm and interpreted using standard zone of inhibition charts.

Result and Discussion

A total of 134 foot ulcer patients with Type 2 diabetes presented in this study, which included male 102 (76.1%) and 32 female

(23.9%). The age range was 40–80 years. The duration of the ulcer infection ranged from 1 month to 4 months and the enrolled cases were of Wagner's grade I to III. In this study, 89 (72%) patients were Grade I, 27 (22%) patients were Grade II and 8 (6%) patients were Grade III (Table 1). All swabs were positive for the culture. From the 134 culture positive specimens, 148 isolates were recovered. In that, 120 (89.5%) patients had single organism infection and 14 (10.5%) patients had two organism infections. Single organism infections were in greater percentage than the two organism infection (Table 2). Among the 134 culture positive cases, gram positive organism constituted 58 (40%) cases and the gram negative constituted 85 (57%) cases.

Figure 1 represents the distribution of microorganisms recovered from the pus specimens of diabetic foot ulcer patients. The most common gram positive cocci in order of frequency were *Staphylococcus aureus* (17%), *CONS* (12%), *Streptococcus* spp., (6%) and *Enterococci* spp., (5.0%), while *Escherichia coli* (20%) was the predominant isolate followed by *Pseudomonas* spp., (18%), *Klebsiella* spp.,(10%), *Proteus* spp., (6.0%) and *Acinetobacter* spp., (3%) in gram negative bacilli. Gram negative bacilli accounted to higher numbers than gram positive cocci**.**

The entire gram positive isolates were showed high rate of resistance to ampicillin, clindamycin and methicillin (Table 3). All the gram positive isolates were found to be sensitivity to amikacin and gentamicin (100%) followed by ofloxacin (92.4%), vancomycin (90.1%), ciprofloxacin (79.3%), erythromycin (77.3%), amoxyclav (74.6%), clindamycin (59.7%), methicillin (54.1%) and ampicillin (48.6%) (Figure 2).

The Gram negative isolates *E. coli, Klebsiella* spp., and *Proteus* spp., showed 55.5% to 100% resistance to amoxyclav and ampicillin. *Pseudomonas* spp., showed 100% resistance to ampicillin and 96.2%) to amoxyclav. The entire gram negative bacilli *E.coli, Pseudomonas, Klebsiella* and *Proteus* isolates were showed high rate of resistance to ampicillin, amoxyclav, ciprofloxacin and ofloxacin (Table 4). Gram negative isolates were found to be sensitivity to imipenem (93%) followed by amikacin (89.6%), gentamicin (83.4%), ceftazidime (74.4%), cefotaxime (72.1%), ceftriaxone (71%), ofloxacin (60%), ciprofloxacin (48.1%), amoxyclav (23.7%) and ampicillin (17.5%) Figure 3).

The prevalence of diabetes is increasing in India faster than in any other country in the world. There are about 33 million diabetics mainly from the urban population (Viswanathan, 2007). Diabetes affects many organs of the body but our study was carried out only on the diabetic foot infections of affected patients in Pattukkottai area, Tamilnadu, India.

In this study the majority of people with diabetes are in the 40 to 80 year age range. In developing countries, the majority of people with diabetes are in the 45 to 64 year age range (King *et al.*, 1998). In this study a total of 148 organisms were isolated from 134 samples averaging 1.1 isolated per culture positive patients. This was nearly similar to study conducted by Banashankari *et al.*, (2012) where culture yielded an average of 1.2. In our study, we have found 89.5% monomicrobial infection. The findings of this study correlate with findings of Jayashree and Sanjeev, (2013).

Among the 148 microorganisms were isolated, gram positive organisms constituted 58 (40%) cases, the gram negative constituted 85 (57%) cases and *Candida albicans* 5 (3%).

Table.1 General characteristics of diabetic foot patients

Sl. No.	Characteristics	No. of patients (n=134)	Percentage
1.	Age (yrs)- <40	10	7.4%
	40-60	62	46.3%
	60-80	51	38.1%
	>80	11	8.2%
2.	Gender- Male	102	76.1%
	Female	32	23.9%
3.	Type of diabetes mellitus- Type 1	10	7.5%
	Type 2	124	92.5%
4.	Duration of foot infection- <1 month	30	22.4%
	1-2 months	44	32.8%
	2-3 months	38	28.4%
	3-4 months	22	16.4%
5.	Diabetic medication- Oral antidiabetics	52	38.8%
	Insulin	40	29.9%
	Oral antidiabetics and insulin	32	23.8
	None	10	7.5%
6	Grade of ulcer (Wagner)- Type 2 diabetes- 124 Patients		
	Grade 0	-	-
	Grade I	89	72%
	Grade II	27	22%
	Grade III	8	6%
	Grade IV	-	
	Grade V	-	

Table.2 Characteristics of diabetic foot ulcer specimens

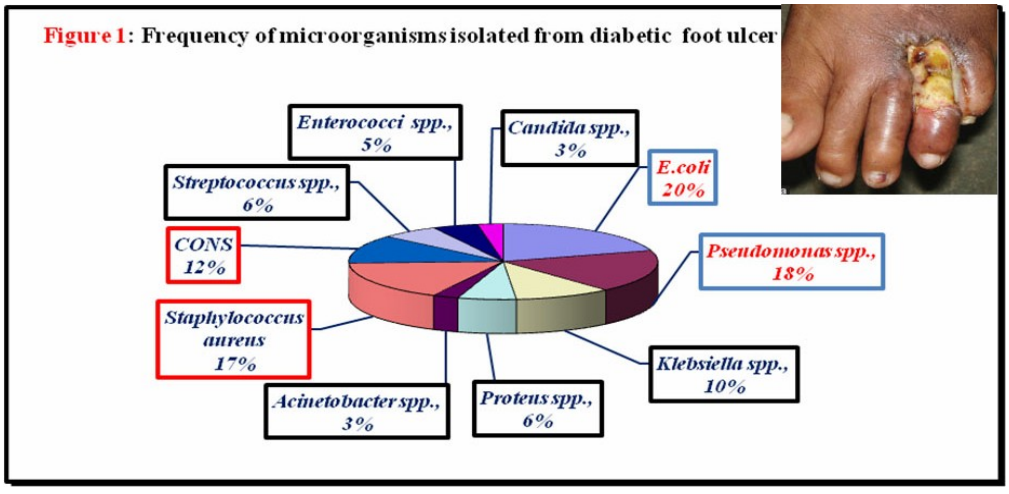
No. of patients	**No. of positive culture**	***Percentage***
No. of patients with positive culture	134	*100%*
Samples with one organism	120	*89.5%*
Samples with two organisms	14	*10.5%*
Gram positive cocci	58	*39.2%*
Gram negative bacilli	85	*57.4%*
Candida albicans	5	*3.4%*
Total No. of isolates	*148*	*100%*

Table.3 Antibiotic resistance pattern of Gram positive organisms

Antibiotics	*Staph. aureus* (N=25)	*CONS* (N=18)	*Streptococcus* spp., (N=9)	*Enterococci* spp., (N=6)
Amikacin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Ampicillin	7 (28.0%)	10 (55.5%)	5 (55.5%)	4 (66.6%)
Amoxyclav	5 (20.0%)	5 (27.7%)	2 (22.2%)	2 (33.3%)
Clindamycin	7 (28.0%)	5 (27.7%)	5 (55.5%)	3 (50%)
Ciprofloxacin	4 (16.0%)	2 (11.1%)	2 (22.2%)	2 (33.3%)
Erythromycin	6 (24%)	2 (11.1%)	2 (22.2%)	2 (33.3%)
Gentamicin	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Methicillin	7 (28.0%)	9 (50.0%)	5 (55.5%)	3 (50%)
Ofloxacin	2 (8.0%)	2 (11.1%)	1 (11.1%)	0 (0%)
Vancomycin	3 (12%)	2 (11.1%)	0 (0%)	1 (16.6%)

Table.4 Antibiotic resistance pattern of Gram negative organisms

Antibiotics	*E.coli* (N=30)	*Pseudomonas* spp., (N=27)	*Klebsiella* spp., (N=15)	*Proteus* spp., (N=9)
Amikacin	4 (13.3%)	4 (14.8%)	2 (13.3%)	0 (0%)
Ampicillin	25 (83.3%)	27 (100%)	12 (80.0%)	6 (66.6%)
Amoxyclav	22 (73.3%)	26 (96.2%)	12 (80.0%)	5 (55.5%)
Ceftazidime	5 (16.7%)	10 (37.0%)	4 (26.6%)	2 (22.2%)
Ciprofloxacin	15 (50.0%)	18 (66.6%)	7 (46.6%)	4 (44.4%)
Ceftriaxone	9 (30.0%)	10 (37.0%)	4 (26.6%)	2 (22.2%)
Cefotaxime	9 (30.0%)	7 (25.9%)	5 (33.3%)	2 (22.2%)
Gentamicin	7 (23.3%)	5 (18.5%)	2 (13.3%)	1 (11.1%)
Imipenem	2 (6.6%)	4 (14.8%)	1 (6.6%)	0 (0%)
Ofloxacin	10 (33.3%)	18 (66.6%)	4 (26.6%)	3 (33.3%)



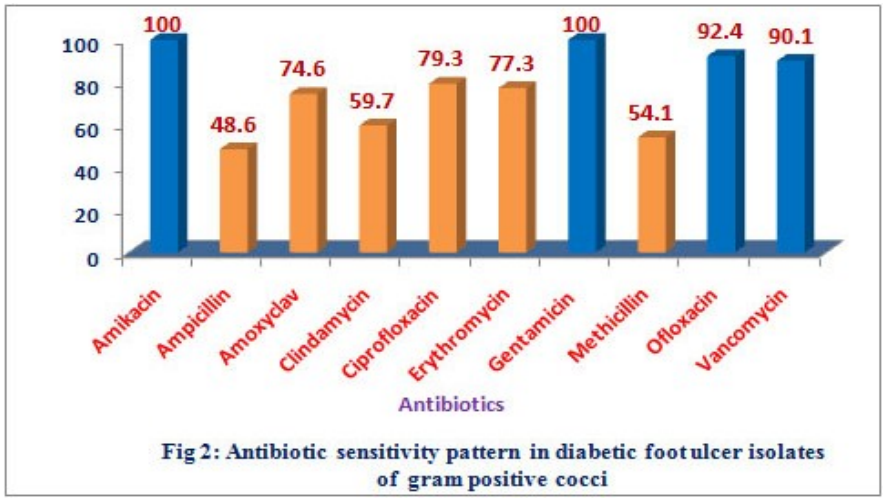


Fig 2: Antibiotic sensitivity pattern in diabetic foot ulcer isolates of gram positive cocci

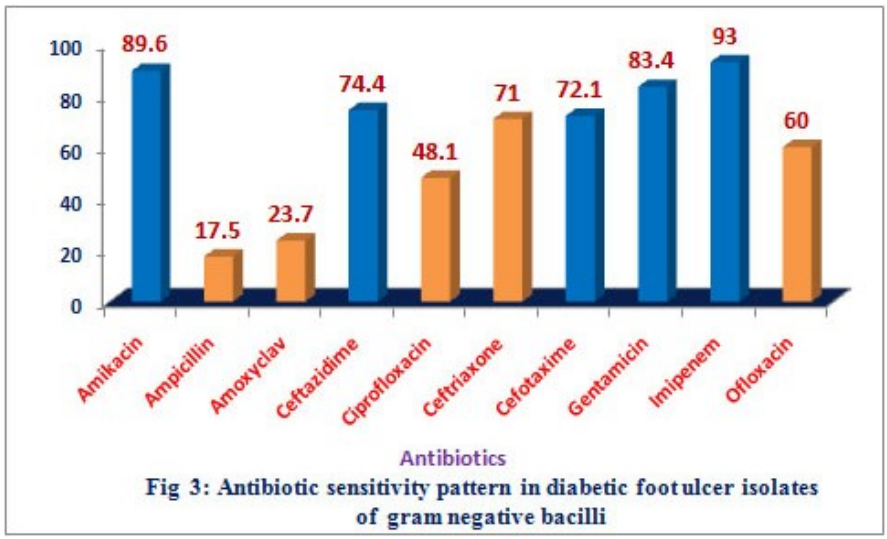


Fig 3: Antibiotic sensitivity pattern in diabetic foot ulcer isolates of gram negative bacilli

Although in gram positive organism *Staphylococcus aureus* (17%) was the predominant isolate followed by *CONS* (12%), while *Escherichia coli* (20%) was the predominant isolate followed by *Pseudomonas* spp., (18%) in gram negative organism. Gram negative organisms accounted to higher numbers than gram positive organisms. The findings of the study are similar to the reported by Mehta *et al.* (2014). The overall prevalence of foot ulcer in male was 76.1%. This result is similar to those reported from many other centers (Ananthi *et al.*, 2004).

The antimicrobial susceptibility pattern showed ofloxacin, vancomycin, ciprofloxacin, erythromycin and amoxyclav as effective antibiotics against gram positive organism while amikacin, cefotaxime, ceftazidime, ceftriaxone, gentamicin and imipenem as effective antibiotic against gram negative organism. This result was similar to the reported by Tiwari *et al.* (2012) and Banoo *et al.* (2012). Antibiotic susceptibility pattern of *Acinetobacter* and *Candida* species was not included due to the fact that they did not meet the minimum number of isolates as per recommendation in

the M39-A4 manual (CLSI, 2010). The limitation of this study was that anaerobes were not isolated. This is probably due to lack of culture media facilities.

Many organisms showed multi-drug resistance. This increasing incidence of multi-drug resistant organisms is a potential risk factor in management of diabetic foot infections which may lead to devastating complications like systemic toxicity, gangrene formation and amputation of lower extremity. Nowadays combination of drugs shows successful remedies for the treatment of diabetic foot infections.

Conclusion

This study showed most common organisms present in the diabetic foot ulcer were Gram-negative aerobes. However, *S. aureus* and *E. coli* was the most predominant organism isolated from the lesions. Most of them our patients were of grade 2 and 3 ulcers according to Wagner grade. In this study ciprofloxacin, ofloxacin and vancomycin were found to be sensitive for the gram positive bacteria. For the gram negative bacilli imipenem, amikacin, gentamicin and ceftazidime were effective. Presence of MDR organisms was alarmingly high in the diabetic foot ulcers. These observations are important, especially for patient management and the development of antibiotic treatment guidelines. Appropriate usage of antibiotics based on local antibiogram pattern can certainly help the clinician in reducing the burden of diabetic foot infections, which ultimately reduces the rate of amputations.

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