Research Article

Spectrum of microbiota in diabetic foot infections in a teaching hospital of Uttar Pradesh

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ABSTRACT

Background: Diabetic foot ulcer is the leading cause of non-traumatic lower extremity amputations. A prospective case–control study was carried out on 50 patients with diabetic foot ulcers (cases) and 50 with non-diabetic foot ulcers (controls) to determine the microbiological profile, their antibiotic sensitivity patterns along with different demographic parameters. The prevalence of extended-spectrum beta-lactamases (ESBLs) producers, carbapenemase producers, and methicillin-resistant *Staphylococcus aureus* among the isolates and the potential risk factors for infection of ulcers with these multidrug-resistant organisms (MDROs) was also studied along with the outcome of these infections. **Objectives:** The present study aims at identifying the microbiological spectrum in these diabetic foot infections and their antibiotic sensitivity pattern in a developing country, like India. **Materials and Methods:** Clinical specimens were taken from each patient with diabetic and non-diabetic foot ulcers. These specimens were processed for culture (aerobic and anaerobic), further identification and antibiotic sensitivity testing. ESBL and carbapenemase production was detected by phenotypic methods and automated VITEK-2 system, and the results were mutually compared by the two methods. **Results:** A total of 158 organisms (155 bacteria and 3 fungi) were isolated giving an average of two organisms/patient. Cases were mostly positive for polymicrobial growth (77%) as compared to controls, and tissue was the most yielding sample. Gram-negative aerobes were predominantly isolated, followed by Gram-positive aerobes. Anaerobic Gram-negative (3%) and fungal (3%) isolates were also seen in the case samples. **Conclusion:** *Escherichia coli* among the Gram-negative (33%) and *S. aureus* among the Gram-positive (7%) were the predominantly isolated organisms, while *Candida* was the most predominantly isolated fungus. A strong association of peripheral vascular disease (PVD), smoking ($P = 0.001$), as well as neuropathy ($P < 0.001$) was seen in the case samples ESBL (44%) and carbapenemase (37%) producers were significantly more in cases as compared to controls. CTX-M like was the most common ESBL phenotype of the isolates. One isolate (3.2%) was detected as resistant due to AmpC enzyme by the VITEK-2 system and four were also positive for metallo-B-Lactamase production. MDRO infection was associated with the presence of neuropathy, PVD, ulcer size >5 cm^2, and osteomyelitis ($P<0.01$).

KEY WORDS: Carbapenemase Production; CTX-M Phenotype; Diabetic Foot Ulcer; Phenotypic Methods, VITEK-2

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INTRODUCTION

Diabetic foot infection is an infection, often originating from an ulcer that occurs in a patient with diabetes mellitus. It heals slowly, can progress, and is associated with high morbidity and serious complications (e.g., osteomyelitis, gangrene, and amputation). Clinical presentation varies widely, depending

on extent and duration of infection, and patient's degree of sensory impairment. A patient with diabetes has almost 25% lifetime risk of developing foot ulcer.[1]

Local features include purulence, erythema, induration, tenderness, or calor it may be accompanied by systemic indicators of infection such as fever or hypothermia, tachycardia, or tachypnea. Diagnosis is clinical, based on the presence of local and systemic signs and symptoms of inflammation.

Patients at highest risk for infection lack the ability to perceive it because they often have sensory neuropathy and retinopathy; family members or other caretakers must be vigilant on patient's behalf for signs of foot injury and infection. Infection often leads to hyperglycemia and may precipitate diabetic ketoacidosis or other metabolic derangements.[1]

Aggressive wound care is essential, beginning with surgical debridement. Offloading of pressure is critical to healing, but it must allow frequent wound inspection and dressing changes until infection clears.[2] Prognosis is guarded; about 20% of moderate-to-severe infections require amputation; in the remainder, healing is often very slow and/or incomplete.

Treatment includes antibiotic therapy and wound care. Antibiotic therapy is recommended for all patients; selection of empiric regimens is based on the severity of infection and risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) and *Pseudomonas aeruginosa*. The present study aims at identifying the microbiological spectrum in these diabetic foot infections and their antibiotic sensitivity pattern in a developing country, like India.

MATERIALS AND METHODS

Sample Size Calculation

$Z^2pq/d^2 = 43$ ($P = 80\%$, q = 1-p, d = 0.12, Z =1.96).

A prospective case–control study was carried out on 100 patients during August 2015–July 2016 at King George's Medical University, Lucknow. 50 patients with diabetic foot ulcer were enrolled as cases (Ulcer grade 2–3 was mainly included) and the rest with non-diabetic foot ulcers as controls. Consent was taken from every patient. The study was approved by the institute's ethical committee (Ref.code: 75th ECM II-B-Thesis-P10).

Meggit-Wagner's classification[3] was used for grading the foot ulcers. Different demographic parameters such as age, sex, type and duration of diabetes, ulcer size and duration, glycemic control during the hospital stay, presence of nephropathy (S. creatinine > 1.8), neuropathy, peripheral vascular disease (PVD), hypertension, obesity, clinical outcome, and duration of hospital stay were noted for each patient.

Clinical, radiographic, and intraoperative signs were used for diagnosing osteomyelitis in the patients.

Microbiological Processing

Samples were taken from each patient with diabetic and non-diabetic foot ulcers. Samples were mainly pus aspirated from wound or bone (osteomyelitis) or tissue debrided from infected wound.

The samples were processed in the microbiology laboratory for both aerobic and anaerobic isolates, followed by identification and antibiotic sensitivity testing. Organisms were identified by routine laboratory methods and biochemical tests.[4]

Antibiotic Sensitivity Testing

Kirby–Bauer disc diffusion method was used for different bacterial isolates[5] and by disc diffusion method for various yeasts[6] isolated from the samples. Staphylococcal isolates were tested for methicillin resistance by measuring the zone diameters using cefoxitin discs. All the anaerobic isolates were tested with discs of colistin, imipenem, and metronidazole. Aerobic Gram-negative bacilli were tested for extended-spectrum beta-lactamases (ESBLs) production by Clinical Laboratory Standards Institute disc diffusion method[5] (screening and confirmation). Modified Hodge test[5] was used for testing the carbapenemase producers. Phenotypic detection of metallo-b-lactamase production was done using imipenem and imipenem + ethylenediaminetetraacetic acid disc[9] combination and observing the zone diameters. AmpC production was also seen in the enterobacteriaceae by testing the isolates for zone diameters with cefoxitin disc alone and in combination with cloxacillin disc.[7,8] The results were compared with the automated VITEK-2 system.[10]

Multidrug-resistant organisms (MDROs) in the present study were defined as MRSA, ESBL, and carbapenemase producers.

Statistical Analysis

SPSS software was used for analyzing the data. Qualitative variables were expressed as percentages, while quantitative variables as means ± standard deviation. The association of demographic parameters with cases and control patients was tested using Student's *t*-test or Fischer's exact test as appropriate. Study variables were also tested in MDRO and non-MDRO infections. $P < 0.05$ was taken as statistically significant. Independent predictors of MDRO infections were assessed using multiple logistic regressions and odds ratio was also calculated (with 95% CI) for having MDRO-infected ulcers.

RESULTS

Male preponderance was seen in the samples for both the cases (86%) and controls (74%). The mean age of the subjects was 48 ± 10.2 years. In the present study, Type II (63%) patients were much more affected with diabetic foot as compared to Type I, and the mean duration of diabetes was 8 ± 2.1 years. Wagner's Grade III (46%) ulcer was the most commonly involved type, and TOES (33%) with the left side of the body (48%) was the most affected site with diabetic foot ulcers in the study. Grade II socioeconomic status (24.48%) was the most commonly involved group among the patients.

PVD, neuropathy, and smoking had a significant association with the development of diabetic foot ulcers.

Of 100 specimens, growth was seen in 100% of the samples in which total 158 organisms were isolated and the average was of 1.58 organisms per patient. Gram-negative rods (83.5%) were the predominant isolates in both the case and control samples. The spectrum of the organisms isolated is depicted in Table 1.

Of the total, 158 isolates, 96.2% were aerobic, 2% were anaerobic, and rest 2% were fungal isolates. The ratio of Gram-negative to Gram-positive was 5.7:1.0 (83.5% vs. 14.5%). There were a total of three anaerobic isolates, of which one was *Veillonella* (Gram-negative cocci) and two were *Bacteroides* spp. (Gram-negative rods). Two isolates, one each of *Stenotrophomonas maltophilia* and *Aeromonas salmonicida* (identified by VITEK-2 system) were also identified.

Polymicrobial etiology was seen in the maximum samples of cases (43) as compared to controls, while control samples were mainly positive for monomicrobial growth (42). It clearly indicated the "polymicrobial" nature of diabetic foot infections. The tissue samples yielded maximum isolates in our study.

The results of susceptibility studies are summarized in Tables 2 and 3.

Gram-positive isolates in cases were mostly sensitive to vancomycin, levofloxacin, gentamicin, amikacin, and tetracycline while they were mostly resistant to amoxyclav, erythromycin, ciprofloxacin, and cotrimoxazole. Two isolates were identified as MRSA (33.3%) and one as vancomycin-resistant enterococci (16.7%) among the isolates. Gram-negative isolates in cases were mostly sensitive to cefoperazone-sulbactam, levofloxacin, colistin, aztreonam, and tetracycline.

On comparing between the two groups, ESBL and carbapenemase producers were much more common among cases as compared to controls [Figure 1]. *Escherichia coli* was the most common isolate for ESBL and carbapenemase

Table 1: Distribution of organisms on the basis of Gram's staining

Organisms	Gram's reaction	Organisms	Number of isolates (%)	
			Cases (97)	Controls (58)
Aerobic bacterial isolates	Gram-positive	*Staphylococcus aureus*	6 (6)	4 (6.8)
		CONS	2 (2)	0
		Enterococcus spp.	6 (6)	2 (3.4)
	Gram-negative	*Escherichia coli*	27 (27)	25 (43.1)
		Pseudomonas aeruginosa	18 (18.5)	12 (20.6)
		Klebsiella pneumonia	3 (3)	1 (1.72)
		Proteus spp.	11 (11.3)	3 (5.17)
		Acinetobacter spp.	7 (7.1)	4 (6.8)
		Enterobacter spp.	1 (1)	2 (3.4)
		Citrobacter spp.	8 (8.2)	5 (8.6)
		Stenotrophomonas maltophilia	1 (1)	0
		Aeromonas salmonicida	1 (1)	0
Anaerobic bacterial isolates	Gram-positive	-	-	-
	Gram-negative	*Veillonella*	1 (1)	0
		Bacteroides	2 (2)	0
Fungal isolates		*Candida albicans*	2 (2)	0
		Candida tropicalis	1 (1)	0

Table 2: Antibiotic resistance pattern of Gram-positive organisms (cases)

Organism	Total No. of isolates	Antibiotics (% of resistance)												
		E	CD	Cx	Va	Te	G	Ak	Le	Cip	HLG	AMC	AMPI	COT
Staphylococcus aureus	6	3 (50)	3 (50)	2 (33.3)	0	1 (16.7)	1 (16.7)	0	2 (33.3)	3 (50)	-	4 (66.7)	-	3 (50)
CONS	2	2 (100)	2 (100)	1 (50)	0	0	2 (100)	1 (50)	1 (50)	2 (100)	-	2 (100)	-	1 (50)
Enterococcus spp.	6	4 (66.7)	-	-	1 (16.7)	2 (33.3)	-	-	3 (50)	4 (66.7)	2 (33.3)	-	4 (66.7)	-

E: Erythromycin, CD: Clindamycin, Cx: Cefoxitin, Va: Vancomycin, Te: Tetracycline, G: Gentamicin, Ak: Amikacin, Le: Levofloxacin, Cip: Ciprofloxacin, HLG: High-level gentamicin, AMC: Amoxyclav, AMP: Ampicillin, COT: Cotrimoxazole

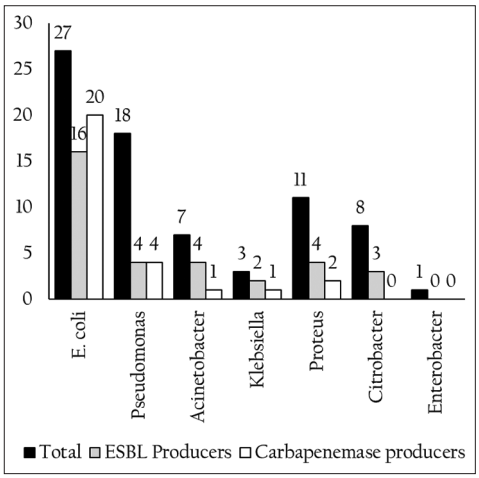

Figure 1: Percentage of extended-spectrum beta-lactamases and carbapenemase producers among different isolates in cases

production both in cases and controls. Pseudomonas was the next predominant ESBL and carbapenemase producer.

No significant differences were seen in age, sex, and duration of diabetes. MDROs were more likely to be associated with an ulcer of size >5 cm^2 (OR 11.2, $P = 0.001$). Neuropathy (3.82, $P = 0.02$) and osteomyelitis (3.42, $P = 0.01$) were present more frequently in MDRO ulcers.

A strong significant association of PVD was seen with MDRO (3.52, $P = 0.01$) [Table 4]. Surgical treatment (5.12, $P < 0.01$) was done in most of the patients with MDRO infections. The presence of neuropathy and ulcer size correlated very well as was seen by multiple logistic regressions, hence, proving that MDRO infections are mostly seen with an ulcer size >5 cm^2 and neuropathy.

DISCUSSION

The present study presents a comprehensive as well as a comparative clinical and microbiological spectrum of diabetic and non-diabetic foot ulcers in the hospitalized patients. An average of 1.58 organisms per patient was isolated from 100 patients. This is similar to the findings of Bansal *et al.*,[11] where culture specimens yielded a mean of 1.52 organisms per sample. Chicholikar *et al.*[12] have also reported an average of 1.3 organisms per sample. Gram-negative organisms were predominantly found in our study, which is in accordance with the findings of Shankar *et al.*[13] *E. coli*, Gram-negative (27%) and *S. aureus*, Gram-positive (6%), were the most commonly isolated pathogens, while the prevalence of other organisms such as *Klebsiella pneumonia*, *P. aeruginosa*, and *Proteus* sp. was 3%, 18%, and 11%, respectively, in the case samples.

Almost similar results were obtained by Chincholikar *et al.*[12] (*P. aeruginosa* [19%], *Klebsiella pneumoniae* [18%], *E. coli* [15%], and *Proteus* sp. [9.3%]), though they reported highest positivity of *S. aureus* (31%) in their study. Ramani *et al.*[14] and Prabhakar *et al.*[15] also made similar observations. One isolate each of *S. maltophilia* and *A. salmonicida* was also identified in the case samples. In the present study, *A. salmonicida* was isolated from a patient of West Bengal who was a fisherman by occupation. He gave the history of frequent exposure to seawater, thereby proving the high chances of isolation of this particular species. *A. salmonicida* is a fish pathogen and is Gram-negative, facultative anaerobic bacteria that occur ubiquitously in aquatic environments. Many of the systemic infections arise following contamination of lacerations and fractures with aeromonas-rich waters. In the present study, *Candida* sp. was isolated only among the cases, with *Candida albicans* being the most common species, followed by *Candida tropicalis*. Similarly, Chincholikar *et al.*[12] reported the high prevalence of *Candida*. Chakarbarti *et al.*[16] too have reported *C. tropicalis* as the predominant isolate. ESBL production was seen in 16 (59.2 %); out of the 27 *E. coli* which were isolated. Carbapenem resistance was seen in 20 (74 %) isolates. *E. coli* was the highest ESBL and carbapenemase producer. Gadepalli *et al.*[17] documented that *E. coli* was the second highest ESBL producer in their study of the 18 pseudomonas isolates, four were carbapenemase producers as per the modified Hodge test which were further identified as metallo-b-lactamase producers both by phenotypic and by VITEK-2 system. These findings correlate with our study. Shanker *et al.*[13] have reported that 44% of the *Pseudomonas* isolates were multidrug resistant. All the fungal isolates were 100% sensitive to Voriconazole and resistant to fluconazole. 50% of the *C. albicans* isolates were sensitive to amphotericin B, whereas *C. tropicalis* was resistant to both amphotericin B and fluconazole. Similar findings have been reported by Chakrabarti *et al.*[16] and Goswami.[18]

Three anaerobic spp. were isolated, among which *Bacteroides* spp. was the most common isolate. Similar findings have

Table 3: Antibiotic resistance pattern of Gram-negative organisms (cases)

Organism	Total number of isolate	Antibiotics (% of resistance)														
		AMC	CTR	CPM	G	AK	Te	P/T	IMI	Le	Cip	AT	MRP	CAZ	PB	CL	CFS
Escherichia coli	27	24 (88.8)	24 (88.8)	23 (85.1)	15 (26.3)	18 (66.6)	12 q (44.4)	16 (59.2)	16 (59.2)	18 (66.6)	20 (74)	12 (44.4)	18 (66.6)	-	-	0	14 (51.8)
Pseudomonas aeruginosa	18	16 (88.8)	-	16 (88.8)	11 (61.1)	9 (50)	6 (33.3)	7 (38.8)	6 (33.3)	11 (61.1)	11 (61.1)	5 (27.7)	8 (44.4)	11 (61.1)	-	2 (11.1)	6 (33.3)
Klebsiella pneumonia	3	3 (100)	3 (100)	3 (100)	2 (66.6)	3 (100)	1 (33.3)	2 (66.6)	1 (33.3)	2 (66.6)	3 (100)	0	2 (66.6)	-	-	0	1 (33.3)
Proteus spp.	11	9 (81.8)	9 (81.8)	9 (81.8)	4 (36.3)	6 (54.5)	-	3 (27.2)	5 (45.4)	6 (54.5)	7 (63.6)	3 (27.2)	8 (72.7)	-	-	0	5 (45.4)
Enterobacter spp.	1	0	0	0	1 (100)	0	0	0	0	0	0	0	0	-	-	0	0
Citrobacter spp.	8	7 (87.5)	6 (75)	6 (75)	4 (50)	5 (62.5)	1 (12.5)	4 (50)	4 (50)	3 (37.5)	6 (75)	0	4 (50)	-	-	0	1 (12.5)
Acinetobacter spp.	7	6 (85.7)	6 (85.7)	6 (85.7)	4 (57.1)	5 (71.4)	0	4 (57.1)	3 (42.8)	4 (57.1)	5 (71.4)	1 (14.2)	4 (57.1)	-	-	0	2 (28.5)
Stenotrophomonas maltophilia	1	1 (100)	1 (100)	1 (100)	0	0	0	0	0	0	1 (100)	0	0	-	-	-	0
Aeromonas salmonicida	1	1 (100)	0	1 (100)	0	0	0	0	0	0	1 (100)	0	1 (100)	-	-	0	0

AMC: Amoxyclav, CTR: Ceftriaxone, CPM: Cefepime, G: Gentamicin, Ak: Amikacin, Te: Tetracycline, P/T: Piperacillin-tazobactam, IMI: Imipenem, Le: Levofloxacin, Cip: Ciprofloxacin, AT: Aztreonam, MRP: Meropenem, CAZ: Ceftazidime, PB: Polymyxin B, Cl: Colistin, CFS: Cefoperazone-sulbactam

been reported by Ramani *et al.*[14] and Criado *et al.*[19,20] As regards the antibiotic sensitivity pattern of anaerobic organisms, all (100%) were sensitive to metronidazole, colistin, and imipenem. These results are in agreement with Prabhakar *et al.*[15] where they have reported that all the anaerobic organisms were found sensitive to metronidazole and chloramphenicol. Furthermore, Ramani *et al.*[14] in their study have found all the anaerobic isolates sensitive to metronidazole. Smoking, PVD, and neuropathy were seen as possible risk factors for the development of ulcers in the cases. Patients with a history of smoking were much more prone to develop ulcers as compared to non-smokers. Ischemia due to the PVD of the lower limbs is another contributory factor in the pathogenesis of the diabetic foot problems.

The present study confirmed that MDRO infection was extremely common in hospitalized patients with diabetic foot ulcers. This is in accordance with the report of Heurtier *et al.*[21] Almost 70% of our patients were infected with MDROs in our study automated VITEK-2 system gave a detection rate of 41.3% for ESBL and 37% for carbapenemase. VITEK-2 in our present study gave concordant results with the phenotypic methods for nearly 98% of the isolates. Teresa *et al.*[10] also quoted similar results in their study which showed that the ESBL classification furnished by the VITEK-2 ESBL test system was concordant with that of the comparison method (molecular identification of beta-lactamase genes) for 99.3% of the isolates evaluated.[10] It was noted that CTX-M like was the most common ESBL (61%) in our present study. In the current study too, only one isolate (3.2%) was detected as resistant due to AmpC enzyme; whereas, four isolates were positive for metallo-B-lactamase production in *Pseudomonas* spp.

Majority of our patients healed by conservative treatment in around 10–12 weeks, while only a minority had to undergo amputation.

CONCLUSION

E. coli among the Gram-negative (33%) and *S. aureus* among the Gram-positive (7%) were the predominantly isolated organisms, while *Candida* was the most predominantly isolated fungus. A strong association of PVD, smoking ($P = 0.001$), as well as neuropathy ($P < 0.001$) was seen in the case samples. ESBL (44%) and carbapenemase (37%) producers were significantly more in cases as compared to controls. CTX-M like was the most common ESBL phenotype of the isolates.[22] One isolate (3.2%) was detected as resistant due to AmpC enzyme by the VITEK-2 system and four were also positive for metallo-B-lactamase production. MDRO infection was associated with the presence of neuropathy, PVD, ulcer size > 5 cm^2, and osteomyelitis ($P < 0.01$).

Table 4: Study parameters in cases with MDROs and non-MDROs infection

Demographic parameters	Non-MDRO	MDRO	P-Value	OR (95% CI)
n (sample size)	14	36		
Age (years)				
<50	2 (17.5)	11 (31.3)		1.00
51–60	6 (45.3)	14 (38.0)	0.47	0.48 (0.14–1.76)
>60	6 (36.2)	11 (31.6)		0.52 (0.16–1.88)
Sex				
Male	11 (81.2)	31 (86.5)		1.00
Female	3 (19.0)	5 (14.0)	0.71	0.76 (0.47–1.06)
Duration of diabetes (years)				
<10	4 (28.5)	13 (37.0)		1.00
10–19	8 (55.5)	19 (53.0)		0.68 (0.32-2.56)
>20	2 (18.2)	4 (11.1)	0.48	0.38 (0.09–1.76)
Type of diabetes				
Type 1	3 (23.0)	2 (7.0)		1.00
Type 2	11 (78.5)	34 (94.4)	0.19	3.98 (0.98–16.57)
Duration of ulcer (months)				
<3	9 (64.0)	29 (79.8)		1.00
>3	5 (35.7)	7 (19.4)	0.21	0.51 (0.28–1.52)
Size of ulcer (cm^2)				
<5	8 (59.0)	4 (12.1)		1.00
>5	6 (42.8)	32 (88.8)	0.001	11.2 (4.30–34.32)
Wagner's classification of grade of ulcer				
1	1 (100.0)	0		-
2	5 (37.5)	8 (62.5)		1.00
3	-	23 (100.0)		-
4	2 (20.0)	8 (80.0)		3.31 (0.82–7.32)
5	-	3 (100.0)	0.05	-
Hypertension	14 (100.0)	26 (72.0)	0.01	-
PVD	6 (41.0)	25 (71.0)	0.01	3.52 (1.32–9.86)
Nephropathy (S. creat>1.8)	10.2 (73.0)	27 (76.0)	0.68	1.06 (0.32–3.42)
Neuropathy	10 (73.0)	33 (92.0)	0.02	3.82 (0.98–13.72)
Associated osteomyelitis	6 (40.9)	26 (70.9)	0.01	3.42 (1.32–8.76)
Treatment				
Medical	8 (57.0)	7 (19.4)		1.00
Surgical	6 (43.0)	29 (80.5)	<0.01	5.12 (1.74–13.76)
Outcome				
Ulcer healed	14 (100.0)	23 (63.7)		-
Amputation	0 (0.0)	13 (36.1)	0.42	-

PVD: Peripheral vascular disease

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