Clinico-microbiological profile and evaluation of antimicrobial sensitivity pattern of diabetic foot ulcers in India

Authors (clockwise from top left): *Shirsendu Mondal, Manasi Banerjee, Sangeeta De and Dipak Kumar*

Diabetic foot ulcers are a serious complication of diabetes mellitus. A cross-sectional observational study was undertaken to determine the antimicrobial sensitivity pattern of common microorganisms found in diabetic foot ulcers in the authors' tertiary care hospital. Of the 50 isolates analysed, multidrug-resistant organisms were present in 36 samples (76.6%). The most common organism isolated was *Pseudomonas aeruginosa*, followed by *Staphylococcus aureus*. Maximum sensitivity was seen with linezolid, followed by vancomycin and teicoplanin.

Shirsendu Mondal *is Assistant Professor;* ***Manasi Banerjee*** *is Associate Professor;* ***Sangeeta De*** *is Postgraduate Trainee;* ***Dipak Kumar*** *is Professor; all at Deptartment Of Pharmacology, Medical College, Kolkata, India*

Diabetes mellitus can be defined as a metabolic disorder characterised by impaired carbohydrate metabolism due to either decreased production of insulin or insulin resistance (American Diabetes Association, 2009). It results in high blood sugar, polyuria (increased production of urine), polydipsia (increased thirst), polyphagia (increased appetite) and various complications, such as diabetic nephropathy, neuropathy and retinopathy (Kharroubi and Darwish, 2015). Globally, an estimated 422 million adults are living with diabetes (World Health Organization, 2016). This number is projected to almost double by 2030 (Wild et al, 2004), and the number of people with diabetes aged 20–79 years is predicted to rise to 642 million by 2040 (Ogurtsova et al, 2017). In 2016, an estimated 1.6 million deaths were directly caused by diabetes. Another 2.2 million deaths were attributable to high blood glucose in 2012 (World Health Organization, 2018).

Three-quarters of people with diabetes live in low- and middle-income families. According to statistics from the International Diabetes Federation, diabetes currently affects over 7.1% of the adult population (Ogurtsova et al, 2017). A study conducted by the Indian Council of Medical Research in 2011 estimated 62.4 million people in India to have diabetes and 77.2 million to be pre-diabetic (Anjana et al, 2011). Type 2 diabetes makes up about 85–90% of all cases of diabetes (Melmed et al, 2011).

As the incidence of diabetes is increasing globally, its related complications are also increasing (Papatheodorou et al, 2016). At some time in their life, 15% of people with diabetes develop foot ulcers that are highly susceptible to infection (Edmonds, 2006; Richard and Schuldiner, 2008). Diabetic foot ulcers (DFUs) are the result of chronic or acute injury to the soft tissues of the foot where there is evidence of pre-existing neuropathy and/or ischaemia, and is a major complication of diabetes (Boulton, 2000). DFU has an estimated prevalence of 4–27% (Nather et al, 2008; Bakri et al, 2012), is a major source of morbidity and a leading cause of hospitalisation in patients with diabetes (Iraj et al, 2013). DFU can lead to infection, gangrene, amputation and even death if necessary care is not provided (Snyder and Hanft, 2009).

Amputation in patients with diabetes is usually precipitated by the development of a chronic wound, which is clinically defined as a wound that fails to heal within 30 days (Falanga, 1998). Colonisation of these wounds, often by multidrug-resistant organisms (MDROs), makes them recalcitrant to healing (Hartemann-Heurtier et al, 2004). MDROs are microorganisms that are resistant to one or more classes of antimicrobial agents. Although the names of certain MDROs describe resistance to only one agent, such as methicillin-resistant *S aureus* (MRSA) or vancomycin-resistant *Enterococci*, these pathogens are frequently resistant to most antimicrobial agents (Siegel

Table 1. Wagner's Classification of Diabetic foot ulcers (Wagner, 1987).

Grade	Description
0	No ulcer in a high-risk foot
1	Superficial ulcer involving the full skin thickness but not underlying tissues
2	Deep ulcer, penetrating down to ligaments and muscle, but no bone involvement or abscess formation
3	Deep ulcer with cellulitis or abscess formation, often with osteomyelitis
4	Localised gangrene
5	Extensive gangrene involving the whole foot

et al, 2007). MDROs are often present in severe diabetic foot wounds. About one-third of patients with a history of previous hospitalisation for their diabetic foot wound and 25% of patients with osteomyelitis were found to have MDRO-positive specimens in one study (Hartemann-Heurtier et al, 2004). For people with diabetes, factors associated with acquiring a MDRO infection include previous antibiotic therapy and its duration, frequency of hospitalisation, duration of hospital stays, osteomyelitis and proliferative retinopathy (Kandemir et al, 2007; Richard et al, 2008).

Although the percentages of the organisms present differs in various studies, the predominant aerobes are *Staphylococcus aureus*, coagulase-negative *staphylococci*, *Streptococcus* species, *Enterococcus* species, *Corynebacterium* species, *Enterobacteriaceae* and *Pseudomonas aeruginosa* (Lipsky, 2008). Most infections are polymicrobial and harbour both aerobes and anaerobes (Lipsky, 2008). An increase in the occurrence of MDRO chronic wound infections in the diabetes population has been noted over the past decade and has been primarily attributed to MRSA, but antibiotic-resistant Gram-negative organisms, particularly *P aeruginosa*, have also been implicated (Falanga, 1998; Synder and Hanft, 2009).

Table 2. Demographic profile of participants.

Demographic	Number (%)
Age (years):	
21–30	2 (4%)
31–40	5 (10%)
41–50	14 (28%)
51–60	18 (36%)
61–70	9 (18%)
71–80	2 (4%)
Gender:	
Male	37 (74%)
Female	13 (26%)
Socioeconomic status:	
Low	39 (78%)
Middle	11 (22%)

Aims

Studies of the bacteriologic profile of DFUs have been performed in various countries (Gadepalli et al, 2006; Citron et al, 2007). Data regarding the sensitivity profile of causative organisms of DFU infections are sparse in the eastern region of India. Thus, the authors aimed to evaluate the antibiotic sensitivity pattern of organisms causing DFU infections in patients presenting at our hospital. As treatment offered to patients is mostly empirical, knowledge about recent trends in the antibiotic sensitivity pattern of organisms commonly present in the region would enable physicians at the authors' hospital to be specific in the choice of antibiotics.

Methods

Study setting

This cross-sectional observational study was carried out over a period of 1 year and 9 months, from November 2015 to August 2017. The study was approved by the Institutional Ethics Committee. A total of 50 patients with DFUs attending the inpatient and out patient surgical departments were enrolled. The participation criteria were the presence of diabetes, a Wagner grade 2 or greater DFU, and evidence of purulent exudate or oedema. Detailed patient histories were collected from all participants.

Sample collection

After cleaning with normal saline, a microbiologist from the hospital swabbed each patient's DFU using sterile cotton moistened with sterile saline. Swabs were taken from sloughy or inflamed tissue, as bacteria tend to be present in greater numbers in these areas. The swabs were placed in sterile glass vials and transported to the microbiology laboratory for culture/sensitivity study.

Chocolate and MacConkey's agars were used for aerobic bacterial culture, and Robertson's cooked meat media/thioglycolate media for anaerobic culture. Aerobic bacterial cultures were incubated at 37°C for 24–48 hours. The colonies obtained were then processed as per standard conventional bacteriological methods (Collee et al, 1996). Kirby-Bauer disc diffusion method was used to test the antimicrobial susceptibility of aerobic isolates as recommended by the 2017 Clinical and Laboratory Standards Institute guidelines (Patel et al, 2017). A subculture from Robertson's cooked meat media

Table 3. Clinical profile of patients and the organisms isolated from their wounds.

Characteristic	Non multidrug-resistant organism	Multidrug-resistant organism	*P*-value
Isolates	11 (23.4%)	36 (76.6%)	–
Age (years):			
<50	3 (27.3%)	10 (27.8%)	1.0
>50	8 (72.7%)	26 (72.2%)	
Gender:			
Male	8 (72.7%)	27 (75.0%)	0.7488
Female	3 (27.3%)	9 (25.0%)	
Duration of diabetes (years):			
<10	5 (45.5%)	13 (36.1%)	
10–19	4 (36.3%)	15 (41.7%)	0.7827
≥20	2 (18.2%)	8 (22.2%)	
Duration of ulcer (months):			
<3	2(18.2%)	5 (13.9%)	0.5634
>3	9(81.8%)	31 (86.1%)	
Size of ulcer (cm²):			
≤4	7 (63.6%)	4 (11.1%)	<0.0001
>4	4 (36.4%)	32 (88.9%)	
Complications:			
Hypertension	7 (63.6%)	11 (30.6%)	0.0765
Retinopathy	0 (0.0%)	2 (5.6%)	–
Nephropathy	6 (54.5%)	8 (22.2%)	0.061
Neuropathy	10 (91.0%)	24 (66.7%)	0.146
Peripheral vascular disease	7 (63.6%)	14 (38.9%)	0.1807
Osteomyelitis	4 (36.4%)	25 (69.4%)	<0.0001

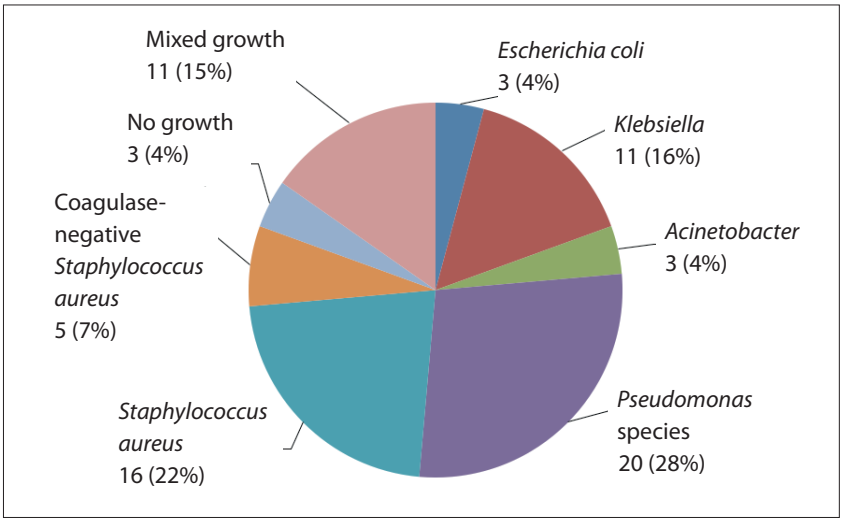

Figure 1. Microbiological profile of diabetic foot ulcers.

was cultured on blood agar and Macconkey's agar, and the plates put in a McIntosh Filde's jar with gas pack for anaerobic incubation. Fungal culture was performed on Sabouraud's dextrose agar.

Clinical factors

Participants' age, sex, clinical signs and symptoms, as well as risk factors were recorded. Ulcer size, including surface area, depth and extent of spread, was measured using Wagner's classification *[Table 1]* (Wagner 1987).

Statistical analysis

Both quantitative and qualitative variables were expressed as percentages. The association of study variables with MDRO and non-MDRO infections was tested by using Fisher's exact test. A two-tailed *p*-value of <0.05 was taken as statistically significant.

Results

Samples from a total of 50 patients' DFUs were analysed. The demographic profile of participants is given in *[Table 2]*. The most common age group presenting with DFUs was 51–60 years. Three-quarters of participants (74%) were male. Most participants (78%) belonged to a low socioeconomic group.

The clinical profile of patients whose DFUs were found to be colonised is shown in *Table 3*. Swabs from a total of 47 out of 50 DFUs had bacterial growth. Of these, MDROs were found to be present in 36 (76.6%) of samples. Older age was associated with the presence of MDROs in DFUs, with 26 (72.2%) patients being 50 years or older. Longer ulcer duration (>3 months) and longer duration of diabetes (>10 years) were both associated with MDRO infections. Neuropathy and osteomyelitis were very commonly associated with DFUs.

The bacterial profile of isolates from infected DFUs is shown in *Figure 1*. In total, 58 organisms were isolated. The most common organism found was *P aeruginosa*, followed by *S aureus*. Gram-positive organisms were present in 36.2% of swab samples and Gram-negative organisms in 63.8% of samples. The antimicrobial sensitivity of the isolated organisms is shown in *Figure 2*. The organisms present were most sensitive to treatment with linezolid, followed by vancomycin and teicoplanin.

Discussion

DFU is the most common complication of diabetes mellitus. It may develop as a result of neuropathy, ischaemia or both, and when infection complicates a DFU the combination can become life-threatening (Khanolkar et al, 2008). Older patients are more likely to present with infected DFUs. In the present study, the mean age of participants was 56 years, which is similar to a study by Ramani et al (1991) in which the mean age of patients with DFU infection was 58 years. The high prevalence of DFU infections in individuals in their late 50s might be due to the occurrence of neuropathy, vasculopathy and altered immune responses, as DFUs are more

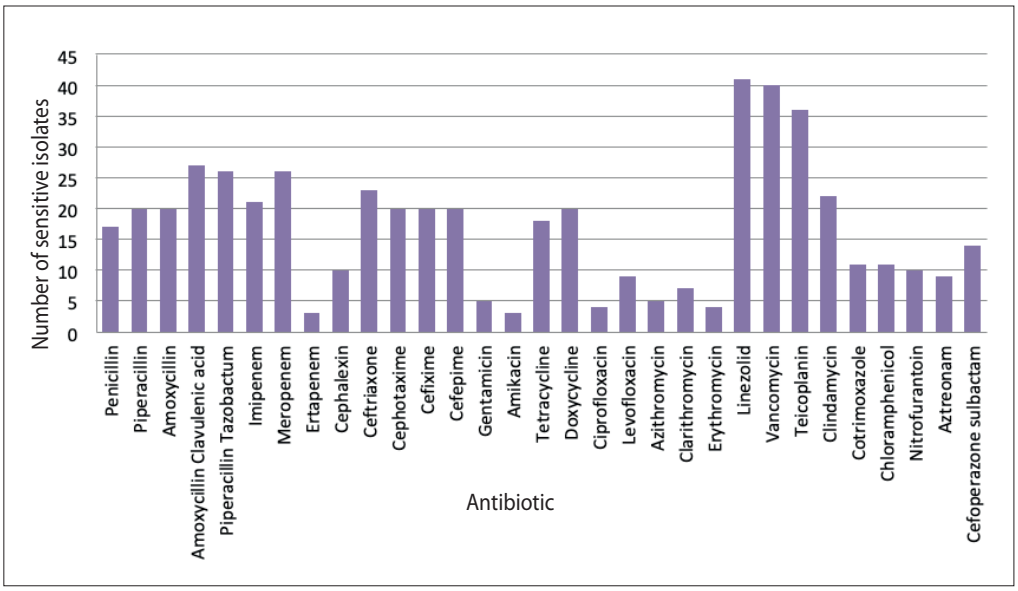

Figure 2. Sensitivity of organisms isolated from diabetic foot ulcers to different antibiotics.

evident as the disease progresses (Ellis Simonsen et al, 2004).

The majority of participants (74%) were male, which could be a result of men spending more time working outdoors, exposing their feet to trauma (Sambashiva Rao and Satyam, 2016). Our observation is comparable with a study by Viswanathan et al (2005) in South Indian patients with diabetes. This finding could also be related to the low socioeconomic class of the majority of participants. The high percentage of trauma seen in this study is, therefore, likely to be the result of a lack of proper hygiene, barefoot walking, low socioeconomic status and lack of access to a proper healthcare system.

The Eurodale studies highlighted peripheral arterial disease and neuropathy as two major risk factors for DFUs (Akhtar et al, 2011). All of the patients in the current study were given a thorough neurological examination with clinical interpretation using vibration sensation, proprioception, pin prick, temperature, reflexes and muscle weakness (Akhtar et al, 2011). Other studies have shown neuropathy to be much more common than vasculopathy in patients with diabetic foot lesions (Ramani and Kundaje, 1990; Viswanathan et al, 2002). In our study, the majority of patients with DFUs containing MDROs had neuropathy (66.7%) and/or osteomyelitis (69.4%). Thus, the presence of neuropathy or osteomyelitis in a patient with an ulcer >4 cm^2 in size appears to be a strong indicator for MDRO infections.

In contrast to some earlier studies showing Gram-positive aerobes to be predominant in diabetic foot infections, we found gram-negative aerobic bacteria to be most frequently responsible. Our results reflect those of similar studies from other parts of India, however, which found Gram-negative bacteria to be more common than Gram-positive bacteria in diabetic foot infections (Shanker et al, 2005; Bansal et al, 2008; Shanmugam et al, 2013).

The most common organisms isolated in our study was *P aeruginosa* (20.3%) followed by *S aureus* (16.2%). Almost two-thirds of patients' DFUs were infected with MDROs. The high rates of antibiotic resistance observed may be due to the fact that the authors' tertiary care hospital serves patients who may have been exposed to broad-spectrum antibiotics before attending hospital, leading to the selective survival of such pathogens. Multidrug-resistant *P aeruginosa* was present in a high number of cases, as has been reported in studies from South India (Shanker et al, 2005).

The isolates in our study showed maximum sensitivity to linezolid, vancomycin and teicoplanin. Linezolid is available in an oral formulation, which results in better patient compliance. Vancomycin and teicoplanin are available in parenteral forms.

The cost of disability, loss of work and lower-extremity amputation extend beyond the economic impact, with regards to patient quality of life. Early appropriate intervention in response to diabetic foot problems is critical to prevent serious complications. Apart from glycaemic control, patient education regarding proper foot care, smoking cessation and self-care form an integral part of measures to prevent the development of foot ulcers. The microbiology of diabetic foot infection is usually polymicrobial in nature and treatment can be challenging, as national and regional antibiotic sensitivity data are lacking. The authors' study aimed to address this pertinent issue. The findings corroborated those from other studies

carried out in India and other countries in terms of clinico-demographic profile, microbiological profile and antibiotic sensitivity pattern. Surveillance of newly-evolving pathogens and antimicrobial resistance is vital in order to decide upon the correct treatment option for the individual patient.

Conclusion

This study provides a rational basis for the selection of empirical drugs in DFU treatment in eastern India. The isolated organisms were most sensitive to linezolid, followed by vancomycin and teicoplanin. Knowledge of microorganism susceptibility should be used to form local treatment guidelines in years to come. DFJME

References

Akhtar S, Schaper N, Apelqvist J, Jude E (2011) A review of the Eurodiale studies: what lessons for diabetic foot care? *Curr Diab Rep* 11(4): 302–9

American Diabetes Association (2010) Diagnosis and classification of diabetes mellitus. *Diabetes Care* 33: S62–7

Anjana RM, Pradeepa R, Deepa M et al (2011) Prevalence of diabetes and prediabetes (impaired fasting glucose and/or impaired glucose tolerance) in urban and rural India: Phase I results of the Indian Council of Medical Research–INdia DIABetes (ICMR–INDIAB) study. *Diabetologia* 54(12): 3022–7

Bakri FG, Allan AH, Khader YS et al (2012) Prevalence of diabetic foot ulcer and its associated risk factors among diabetic patients in Jordan. *Jordan Med J* 46: 118–25

Bansal E, Garg A, Bhatia S et al (2008) Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol* 51(2): 204–8

Boulton AJM (2000) The diabetic foot: a global view. *Diabetes Metab Res Rev* 16: S2–5

Citron DM, Goldstein EJC, Merriam CV et al (2007) Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. *J Clin Microbiol* 45(9): 2819–28

Collee JG, Fraser AG, Marmion BP, Simmons A (1996) *Mackie & McCartney Practical Medical Microbiology.* 14th edn. Churchill Livingstone, New York

Edmonds M (2006) Diabetic Foot Ulcer: Practical treatment recommendations. *Drugs* 66(7): 914–29

Ellis Simonsen SM, Van Orman ER, Hatch BE et al (2004) Cellulitis incidence in a defined population. *Epidemiol Infect* 134(2): 293–9

Falanga V (1998) Wound healing and chronic wounds. *J Cutaneous Med Surg* 3: S1–

Gadepalli R, Dhavan B, Sreenivas V et al (2006) A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. *Diabetes Care* 29(8): 1727–32

Hartemann-Heurtier A, Robert J, Jacqueminet S et al (2004) Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. *Diabet Med* 21(7): 710–5

Iraj B, Khorvash F, Ebneshahidi A, Askari G (2013) Prevention of diabetic foot ulcer. *Int J Prev Med* 4(3): 373–6

John BB, Kenneth S, Polonsky K, Charles FB. Type 2 Diabetes Mellitus. In: Melmed S, Polonsky K, Larsen PR, Kronenberg H (2011) *Williams Textbook of Endocrinology* (12th edn). Elsevier/Saunders, Philadelphia: pp1371–435

Kandemir O, Akbay E, Şahin E et al (2007) Risk factors for infection of the diabetic foot with multi-antibiotic resistant microorganisms. *J Infect* 54(5): 439–45

Khanolkar MP, Bain SC, Stephens JW (2008) The diabetic foot. *QJM* 101(9): 685–95

Kharroubi AT, Darwish HM (2015) Diabetes mellitus: The epidemic of the century. *World J Diabetes* 6(6): 850-867

Lipsky BA (2008) New developments in diagnosing and treating diabetic foot infections. *Diabetes Metab Res Rev* 24(1): S66–71

Nather A, Bee CS, Huak CY et al (2008) Epidemiology of diabetic foot problems and predictive factors for limb loss. *J Diabetes Complications* 22(2): 77–82

Ogurtsova K, Fernandes JDR, Huang Y et al (2017) IDF Diabetes Atlas: Global estimates for the prevalence of diabetes for 2015 and 2040. *Diabetes Res Clin Pract* 128: 40–50

Papatheodorou K, Papanas N , Banach M et al (2016) Complications of diabetes 2016. *J Diabetes Res* 2016: 6989453

Patel JB, Weinstein MP, Eliopoulos GM et al (2017) M100: *Performance Standards for Antimicrobial Susceptibility Testing* (27th edn). Available at: https://bit.ly/2HBtcwc (accessed 29.01.2019)

Ramani A, Kundaje GN (1990) Etiology of diabetic foot ulceration. *J Assoc Physicians India* 38(11): 843–5

Ramani A, Ramani R, Shivananda PG, Kundaje GN (1991) Bacteriology of diabetic foot ulcers. *Indian J Pathol Microbiol* 34(2): 81–7

Richard JL and Schuldiner S. Epidemiology of diabetic foot problems. (2008). Rev Med Interne, 29(2), pp. 222-230.

Richard JL, Sotto A, Jourdan N (2008) Risk factors and healing impact of multidrug-resistant bacteria in diabetic foot ulcers. *Diabetes Metab* 34(4): 363–9

Sambashiva Rao G, Satyam G (2016) A comparative study of diabetic and non-diabetic foot infections with reference to etiopathogenesis, clinical features, and outcome. *Sch J App Med Sci* 4(7): 2389–95

Shanker EM, Mohan V, Premlatha G et al (2005) Bacterial etiology of diabetic foot infections in South India. *Eur J Intern Med* 16(8): 567–70

Shanmugam P, Jeya M, Linda S (2013) The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. *J Clin Diagn Res* 7(3): 441–5

Siegel JD, Rhinehart E, Jackson M, Chiarello L; Healthcare Infection Control Practices Advisory Committee (2007) Management of multidrug-resistant organisms in health care settings, 2006. *Am J Infect Control* 35(10): S165–93

Snyder RJ, Hanft JR (2009) Diabetic foot ulcers-effects on QOL, costs, and mortality and the role of standard wound care and advanced-care therapies. *Ostomy Wound Manage* 55(11): 28–38

Viswanathan V, Jasmine JJ, Snehalatha C, Ramachandran A (2002) Prevalence of pathogens in diabetic foot infection in South Indian type II diabetic patients. *J Assoc Physicians India* 50: 1013–6

Viswanathan V, Thomas N, Tandon N et al (2005) Profile of diabetic foot complications & its associated complications- a multicentric study from India. *J Assoc Physicians India* 53: 935–6

Wagner FW Jr (1987) The diabetic foot. *Orthopaedics* 10(1): 163–72

Wild S, Roglic G, Green A et al (2004) Global Prevalence of Diabetes: Estimates for the year 2000 and projections for 2030. *Diabetes Care* 27(5): 1047–53

World Health Organization (2016) Global Report on Diabetes. WHO, Geneva. Available at: https://www.who.int/diabetes/global-report/en/ (accessed 19.12.2018)