ORIGINAL ARTICLE

A clinicomicrobiological study of diabetic foot ulcers from South Kerala

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ABSTRACT

Background: Infection with multidrug-resistant organisms (MDRO) in foot ulcers is a major cause of morbidity and mortality among diabetic patients in India. The empirical antibacterial therapy based on local prevalence and susceptibility pattern of bacterial isolates can significantly reduce the incidence of complications. **Objectives:** The study aimed to a) determine the microbiological profile and prevalence of MDRO isolated from diabetic foot infection; b) identify the main risk factors for MDRO; and c) find out the risk of a few selected complications in ulcers infected with MDRO. **Materials and Methods:** Specimens such as tissue/bone biopsy or syringe aspirates were collected for culture and antimicrobial sensitivity testing from patients with diabetic foot ulcers of Wagner grade ≥2. Cefoxitin disc diffusion method was used for the detection of methicillin-resistant *Staphylococcus aureus* (MRSA). Standard methods to detect ESBLs, AmpC β-lactamase, and metallo-beta-lactamase were used in multidrug-resistant Gram-negative isolates. **Results:** Out of 250 patients, 144 (58.3%) had polymicrobial infection and 172 (41.7%) of the 412 isolates were multidrug-resistant. *Staphylococcus aureus* was the single commonest organism, i.e., 139 (33.7%). Out of 139 *Staphylococcus aureus* isolates, 62 (44.6%) were MRSA. Gram-negative organisms accounted for the majority of isolates, i.e., 223 (54.1%). Out of 223 Gram-negative organisms, 108 (48.4%) were multidrug-resistant. The commonest Gram-negative bacterium was *Pseudomonas aeruginosa*, i.e., 77/223 (18.7%). Risk factors for MDRO and outcome of MDRO infection were also analysed. **Conclusions:** The prevalence of MDRO in our study was nearly 50%, which shows the urgent need for implementation of strict antibiotic policy and infection control measures to avoid antibiotic resistance. The presence of recurrent ulcer, past hospital stay, past foot-related surgery, ulcer size (>4 cm^2) and ulcer duration >1 week were identified as the main risk factors for MDRO. Death during hospital stay was significantly higher in the MDRO group, while ulcer healing or amputation was not significantly associated. Finally, an empirical antibiotic policy was also proposed for treating diabetic foot infections.

Key words: Diabetic foot infections, multidrug-resistant organisms (MDRO), methicillin-resistant *Staphylococcus aureus* (MRSA)

INTRODUCTION

Diabetic foot infection is a common cause for approaching health care facilities among diabetic patients in India. Infection with multidrug-resistant organisms (MDRO), largely due to the indiscriminate use of antibiotics, causes additional morbidity and mortality. Early diagnosis of microbial infections is aimed to institute appropriate antibacterial therapy based on culture and sensitivity results. However, initial management comprises empirical antimicrobial therapy, which is often based on susceptibility data derived from studies performed on general clinical isolates.

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In view of the above, a prospective microbiological study was carried out at Government Medical College, Thiruvananthapuram, Kerala, India, which is a large referral teaching hospital in South Kerala. This study presents a comprehensive clinical and microbiological survey of patients with infected diabetic foot ulcers admitted to the surgery wards of the Medical College Hospital. The common bacteriologic agents in diabetic foot

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infection were identified and their sensitivity patterns were determined by standard methods. A proposed guideline of empirical drug therapy was derived from the data. It is hoped that compliance to this guideline can contribute to the reduction in the incidence of lower limb amputations in diabetic foot infections.

MATERIALS AND METHODS

This study aimed to do the following:

a. Determine the microbiological profile and prevalence of MDRO isolated from diabetic foot infection.
b. Identify the main risk factors for MDRO.
c. Find out the risk of a few selected complications, in ulcers infected with MDRO.

A prospective study was carried out on 250 diabetic patients with foot ulcer during a 1-year period. Ulcers were graded according to the Wagner classification.[1] The inclusion criterion was patients with diabetes mellitus having clinically infected foot ulcers of Wagner grade ≥2 with or without prior antibiotic therapy. Infection is generally defined clinically by the presence of purulent secretions or at least two of the classic signs of inflammation (redness, warmth, swelling or indurations, tenderness or pain). Diabetic foot lesions of Wagner grades 0 and 1 were excluded from the study.

Method

At the time of admission, the patients were clinically assessed with the help of a *pro forma* which included personal details, clinical history with details of diabetes, glycaemic control and complications, followed by detailed history and examination of the foot ulcer. Simultaneous neurologic (absence of perception of the Semmes-Weinstein monofilament at two of 10 standardized plantar sites on either foot) and vascular evaluation of the foot (ischaemic symptoms with or without absence of pedal pulses) was also done. Osteomyelitis was diagnosed based on x-rays.

Specimens for culture were collected at the time of admission, after the surface of the wound had been washed vigorously by sterile saline followed by debridement of superficial exudates. Curetting/tissue specimens were collected from the deep part of the base of the ulcer using a sterile scalpel blade in a sterile container with sterile solution of normal saline and bone curetting/biopsy for suspected osteomyelitis. Syringe aspirates were taken for abscesses after proper disinfection of skin with 2% povidone iodine solution and 70% ethyl alcohol. Specimens were transported as early as possible, homogenised with sterile mortar and pestle, and processed without delay. Standard methods for isolation and identification of aerobic bacteria were used.

Antibiotic susceptibility testing

Antibiotic sensitivity testing of the isolates was done by the standard disc diffusion method. In the present study, MDRO include methicillin-resistant *Staphylococcus aureus* (MRSA), methicillin-resistant coagulase-negative staphylococci (MRCoNS), betalactamase-producing enterobacteriaceae [extended-spectrum beta-lactamases (ESBLs) and AmpC betalactamases] and non-fermenters which were resistant to three or more classes of antibiotics.

Cefoxitin disc diffusion method was used as per Clinical and Laboratory Standards Institute (CLSI) guidelines for the detection of MRSA. ESBL detection was done for all enterobacteriaceae, which were found to be resistant to third-generation Cephalosporins using double disc synergy method.[2] AmpC beta-lactamases were detected in certain Gram-negative bacteria employing an inhibitor-based test using 3 amino phenyl boronic acid (apb).[3] Screening for metallo-beta-lactamase production was done in imipenem-resistant isolates of *Pseudomonas aeruginosa* by imipenem-ethylenediaminetetraacetic acid (EDTA) combined disc test as described by Young *et al*.[4]

Culture reports were issued and the concerned clinicians were informed about the isolates identified and their antibiotic susceptibility pattern. The patients were followed up till discharge from the hospital and repeat samples were taken as and when necessary. Treatment and outcome were monitored. Ulcers which were completely healed by epithelialisation without any discharge or treated by applying split-skin graft were labeled as cured. The rest of the ulcers were categorised as those with primary healing at the time of discharge, those that remained non-healing and those that underwent amputation.

Statistical analysis

Quantitative variables were expressed as means ± standard deviation, while qualitative variables were expressed as percentages. The associations of study variables with MDRO and non-MDRO infections were tested using the chi-square test. A P value of <0.05 was taken as significant. Odds ratio was also calculated for the study variables.

RESULTS

Demography and clinical factors

As per the criteria, 250 patients admitted to the surgery wards with infected diabetic foot ulcers were studied. Most of patients were in the sixth decade of life, [94 (37.2%)].

The mean age was 57.65 ± 10.5 years. There were 161 (64.4%) males and 89 (35.6%) females, the male:female ratio being 1.8:1. Males were predominantly affected among the study population; only one patient had type 1 diabetes. More than half of the patients (56.4%) were diagnosed to have diabetes mellitus for more than 10 years. The median duration of diabetes was 10 years and the interquartile range was 10 years. Out of the 250 patients, 147 (58.8%) patients had neuropathy, 119 (47.6%) had peripheral vascular disease and 56 (22.4%) had undergone foot-related surgical procedures. Recurrent foot ulcers were seen in 70 (28%) patients, and 23 (9.2%) had osteomyelitis. Notably, 134 (53.6%) had poor glycaemic control (RBS >200 mg/dL) at the time of admission, and 69 (27.6%) had nephropathy. There were 83 (33.2%) anaemic patients [haemoglobin (Hb) <10], 42 (16.8%) hypertensive patients and 32 (12.8%) ischaemic heart disease patients. Symptoms and signs of septicemia were present in 12 (4.8%) patients at the time of presentation. Foot ulcers of Wagner grade 2 were found in 118 (47.2%) patients followed by 76 (30.4%) people with foot ulcers of Wagner grade 4. Grade 3 ulcers were present in 51 (20.4%) patients and only 5 (2%) had grade 5 ulcers. Nearly 57% of the patients had the lesions for more than 1 week.

Microbiological profile

Results were analysed for aerobic bacterial isolates only. Out of 250 patients, 144 (58.3%) had polymicrobial infection and 103 (41.7%) had monomicrobial infection. A total of 412 isolates were detected from the infected ulcers of 250 patients, averaging 1.6 species per patient. Infection due to two organisms occurred in 120 (48%) patients, whereas 24 (9.6%) had infection due to three or more organisms. Three patients (1.2%) had sterile culture. Infection due to Gram-positive organisms alone occurred in 45 (18%) patients, whereas 77 (30.8%) patients had only Gram-negative infection. Out of 250, 125 (50%) had mixed Gram-positive and Gram-negative infections.

Gram-negative organisms accounted for 223 (54.1%) isolates, while 189 (45.9%) were Gram-positive organisms. *Staphylococcus aureus* was the predominant organism isolated, accounting for 33.7% of the isolates. The other common organisms isolated were *Pseudomonas aeruginosa* (18.7%), *Escherichia coli* (12.1%), *Proteus mirabilis* (11.1%) and *Klebsiella pneumoniae* (7.8%). The most common polymicrobial infection was due to MRSA and *Pseudomonas aeruginosa,* followed by *Staphylococcus aureus* and *Escherichia coli*. The antibiotic sensitivity patterns of the isolates are shown in Tables 1 and 2.

Of the total 412 isolates, (41.7%) were multidrug-resistant. MRSA and MRCoNS constituted 64 (33.9%) out of 189 Gram-positive isolates. MRSA constituted 44.6% of the *Staphylococcus aureus* isolates. Out of 223 Gram-negative organisms, 108 (48.4%) were multidrug-resistant.

Table 1: Antibiotic sensitivity pattern of Gram positive isolates – Number (% sensitivity)

Organism (no.)	Pen	Gen	Amp	Ery	Cefox	Amik	Van	Clinda	Rif	Linez
MSSA (77)	5 (6.5)	37 (48.1)	Nt	39 (50.6)	77 (100)	77 (100)	77 (100)	77 (100)	77 (100)	77 (100)
MRSA (62)	0	4 (6.4)	Nt	6 (9.7)	0	54 (87.1)	62 (100)	62 (100)	62 (100)	62 (100)
S.epidermidis (3)	0	1 (33.3)	Nt	1 (33.3)	1 (33.3)	3 (100)	3 (100)	3 (100)	2 (66.7)	3 (100)
Enterococci (25)	0	7 (28)	4 (16)	4 (16)	Nt	7 (28)	25 (100)	Nt	Nt	25 (100)
Streptococcus pyogenes (10)	10 (100)	10 (100)	10 (100)	10 (100)	Nt	10 (100)	10 (100)	Nt	Nt	Nt
Other Streptococci (12)	10 (83.3)	9 (75)	10 (83.3)	10 (83.3)	Nt	10 (83.3)	12 (100)	Nt	Nt	Nt

Pen: Penicillin; Amp: Ampicillin; Gen: Gentamicin; Ery: Erythromycin; Cefox: Cefoxitin; Amik: Amikacin; Van: Vancomycin; Clinda: Clindamicin; Rif: Rifampicin; Llinez: Linezoilid; Nt: Not tested

Table 2: Antibiotic sensitivity pattern of Gram negative isolates – Number (% sensitivity)

Organism	Amp	Gen	Ceph	Cot	Amik	Cefo	Ceft	Cipro	Cefo - sulb	Pip -taz	Imp	Azt	Amox +clav
E.coli (50)	4 (8)	11 (22)	4 (8)	9 (18)	42 (84)	7 (14)	Nt	8 (16)	48 (96)	44 (88)	50 (100)	7 (14)	28 (56)
Klebsiella pneumoniae (32)	0	9 (28.1)	6 (18.8)	5 (15.6)	28 (87.5)	9 (28.1)	Nt	9 (28.1)	30 (93.7)	27 (84.3)	32 (100)	9 (28.1)	20 (62.5)
P.mirabilis (46)	13 (28.2)	15 (32.6)	10 (21.7)	10 (21.7)	36 (78.3)	30 (65.2)	Nt	17 (37)	44 (95.7)	40 (87)	46 (100)	30 (65.2)	36 (78.3)
Pr. vulgaris. (2)	0	0	0	0	2 (100)	0	Nt	0	2 (100)	2 (100)	2 (100)	0	2 (50)
Enterobacter cloacae (1)	0	0	0	0	1 (100)	1 (100)	Nt	0	1 (100)	1 (100)	1 (100)	1 (100)	1 (100)
Citrobacter freundii (1)	0	0	0	1 (100)	1 (100)	0	Nt	0	1 (100)	1 (100)	1 (100)	0	0
P*seudomonas aeruginosa* (77)	Nt	15 (6.7)	Nt	Nt	38 (49.3)	Nt	35 (45.5)	15 (19.5)	52 (67.5)	60 (77.9)	75 (97.4)	52 (67.5)	Nt
Acinetobacter baumanii (14)	0	1 (7.1)	0	2 (14.2)	10 (71.4)	2 (14.2)	Nt	0	9 (64.3)	8 (57.1)	12 (85.7)	2 (14.2)	2 (14.2)

Ceph: Cephalexin; Cot: Cotrimoxazole; Cefo: Cefotaxime; Ceft: Ceftazidime; Cip: Ciprofloxacin; Cefo – sulb — Cefoperazone-sulbactam; Pip-taz: Piperacillin-tazobactam; Imp: Imipenem; Azt: Aztreonam; Amox-clav: Amoxycillin/clavulanic acid

Tables 3 and 4 show the distribution of MDRO and their mechanisms of drug resistance, respectively.

Statistical analysis of risk factors and outcome variables

A univariate analysis of the association of study variables with MDRO infection is presented in Table 5. MDRO-positive status was not associated with patient characteristics (age, sex, type of diabetes, duration of diabetes, complications of diabetes), fever, leucocytosis, glycaemic control, etc. The presence of recurrent ulcer, past hospital stay, ulcer duration more than 1 week and past foot surgery were found to have significant association with MDRO infection. Grade of the ulcer had no significant association with MDRO infection. Patients were followed up till discharge and the following outcome was identified for ulcers. Out of the 250 diabetic foot ulcer patients, 111 (44.4%) had their ulcers cured, 90 (36%) had ulcers that were at the stage of primary healing, 18 (7.2%) had ulcers that were non-healing and 31 (12.4%) had to undergo amputation. None of these parameters were significantly associated with MDRO status, while mortality during hospital stay had significant association. Table 6 shows the distribution of ulcer-related outcome. Table 7 shows the association of outcome variables (patient- and ulcer-related) with MDRO infection.

Table 3: Distribution of multidrug resistant organisms (MDRO) among the isolates

Organism	MDRO No. (%)
Staphylococcus aureus (*n*=50)	62 (44.6)
S.epidermidis (*n*=3)	2 (66.7)
E.coli (n=50)	43 (86)
Klebsiella pneumoniae (*n*=32)	22 (68.8)
Proteus mirabilis (*n*=46)	16 (34.8)
Proteus vulgaris (*n*=2)	2 (100)
Citrobacter freundii (*n*=1)	1 (100)
Pseudomonas aeruginosa (*n*=77)	18 (23.4)
Acinetobacter baumannii (*n*=14)	6 (42.9)
Total (*n*=142)	172 (41.7)

DISCUSSION

In the present study, soft tissues from the ulcer base or bone biopsy samples or syringe aspirates were mainly used to study the microbiology of ulcers as they are more sensitive and specific yielding more pathogens and less contaminants compared to pus swabs. An average of 1.6 organisms per patient is lower compared to the studies by Gadepalli *et al*.[5] probably due to better culture methods for anaerobic isolation. Infection due to both Gram positive and Gram negative organisms in 50% of the patients confirms the necessity to include broad spectrum antibiotics in empirical management of diabetic ulcers. The frequency of Gram negative bacteria was higher than the frequency of Gram positive bacteria, in accordance with a study from South India by Shanker *et al*.[6] and a similar study from North India by Gadepalli et al.

As in our study, the predominance of *S.aureus* and *P.aeruginosa* in diabetic foot infections was encountered by Bansal et al.[7] and Chincholikar *et al*.[8] These findings are again in tune with studies conducted outside India also.[9,10]

Table 4: Distribution of mechanisms of drug resistance among Gram negative isolates No (%)

Resistance mechanism	*E. coli* (*n* = 50)	*K. pneumoniae* (*n* = 32)	*P. mirabilis* (*n* = 46)	*P. vulgaris* (*n* = 2)	*C. freundii* (*n* = 1)	*P. aeruginosa* (*n* = 77)
ESBL	37 (74)	19 (59.4)	13 (28.3)	2 (100)	1 (100)	Nt
AmpC	6 (12)	3 (9.4)	3 (6.5)	0	0	Nt
Metallo betalactamase	Nt	Nt	Nt	Nt	Nt	2 (2.6)

Table 5: Association of study variables in diabetic patients with MDRO and non-MDRO infections

Study variables	MDRO	Non MDRO	Chi square	*P* value	Odds Ratio OR (95% CI)
Neuropathy	79	68	1.235	0.266	1.33 (0.78-2.28)
Nephropathy	38	31	0.696	0.404	1.27 (0.7-2.3)
Peripheral vascular disease	67	52	2.751	0.097	1.52 (0.9-2.6)
Ischaemic heart disease	20	12	2.01	0.156	1.73 (0.76-3.97)
Hypertension	41	34	0.641	0.423	1.25 (0.7-1.44)
Anaemia	49	34	3.72	0.066	1.64 (0.93-2.9)
Poor glycaemic control	75	59	3.089	0.079	1.56 (0.92-2.66)
Osteomyelitis	13	10	0.332	0.565	1.29 (0.54-3.3)
Sepsis	7	5	0.286	0.593	1.38 (0.38-5.16)
Past ulcer	46	24	8.653	0.003	2.34 (1.27-4.34)
Past hospital stay	93	64	12.016	0.001	2.52 (1.44-4.43)
Past foot surgery	36	20	5.251	0.022	2.03 (1.06-3.95)
Ulcer duration (>1 week)	83	59	7.1	0.0081	2.05 (1.19-3.52)
Ulcer size (>4 cm^2)	108	88	6.188	0.013	2.2 (1.12-4.32)
Grade of ulcer			2.63	0.27	

Table 6: Distribution of ulcer related outcome (*n* = 250)

Outcome	No.	Percentage (%)
Cured	111	44.4
Primary Healing	90	36
Remained unhealed	18	7.2
Amputation	31	12.4

Table 7: Association of ulcer and patient related outcome with MDRO infection

Outcome	Non MDRO	MDRO	Chi square	*P* value	Odds ratio
Cured	60	62	0	0.995	1
Primary Healing	34	45	1.75	0.19	1.44
Remained unhealed	11	7	1.1	0.3	0.6
Amputation	18	13	1.11	0.292	0.66
Death	15	3	8.214	0.004	5.35

This study had a lower rate of isolation of nonfermenters like Acinetobacter spp. and Pseudomonas spp.

Among the 250 patients, 127 (50.8%) had MDRO infected ulcers. The prevalence is comparatively less compared to the study conducted by Gadepalli et al. (72%). MRSA has been a pathogen of concern in patients with diabetic foot infection for almost two decades. Indian studies have shown a high prevalence of MRSA upto 56%.[11,12] Such a high prevalence of MRSA is likely to influence the empirical management of diabetic foot infections. A high degree of Ampicillin resistance in enterococci was noted in the present study (84%) compared to other studies (17% by Nadeem et al.).[13] Gram-negative bacteria showed least resistance to imipenem. A high degree of resistance was observed for Ciprofloxacin and Gentamicin.

In the recent years, there has been an increase in the incidence and prevalence of ESBLs. The prevalence of ESBL among Gram-negative isolates is low in our study population compared to that of Gadepalli et al. it is found to be 42/94 (44.7%). Among the predominant isolates of enterobacteriaceae, the highest production of ESBLs was noted in *E. coli* followed by Klebsiella spp. These are contrary to the observation by Gadepalli *et al*., which shows maximum ESBL production in Proteus spp (65.3%). In a study conducted by Varaiya *et al*., 16/31 (51.61%) of *Klebsiella pneumoniae* isolates and 15/31 (48.38%) of *E.coli* isolates were reported to be ESBL producers.[14] Out of the 77 *P.aeruginosa* isolates, only two (2.6%) were found to be metallo betalactamase (MBL) producers and Polymyxin B or Colistin represents the best treatment option. Colistin is very expensive and toxic, hence this limits its use. A study conducted by Varaiya *et al*. revealed 75% resistance to carbapenems amongst *Pseudomonas aeruginosa* isolates from diabetic patients and out of the 33 MBL-producing isolates, 24 (72.7%) were from diabetic patients.[15]

This study could identify significant association of MDRO infection with known risk factors like the presence of recurrent ulcer (OR 2.34), past hospital stay (OR 2.52) and past foot related surgery (OR 2.03). Ulcer duration of more than one week (OR 2.05) and size >4 cm^2 (OR 2.2) were found to be associated with MDRO infection while grade of the ulcer had no significant association. History of chronic antibiotic treatment failed to show any association. This may be because of the inadequate information regarding the type, duration and dosage of antibiotics. MDRO positive status failed to show any association with ulcer healing or amputation. MDRO infection was associated with higher mortality (OR 5.35) compared to non-MDRO infection.

CONCLUSION

This is perhaps the first study of its kind to report the status of MDRO in diabetic foot infections from Kerala. The prevalence of MDRO revealed from this study has to be considered as a warning sign for emerging antibiotic resistance.

Based on the susceptibility data, the following empirical antimicrobial therapies can be considered:

- Life-threatening limb infections can be treated with a combination of Vancomycin and Imipenem.
- Patients with risk factors (given above) for MDRO can be treated with a combination of Linezolid and Piperacillin-tazobactam or Cefoperazone-sulbactam.
- Those without risk factors can be treated with Cloxacillin and Amikacin.

Close follow-up and appropriate changes in the antibiotics based on culture are essential.

The empirical antibiotic policy outlined above can be validated in a clinical setting for its effectiveness.

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Conflicts of interest

There are no conflicts of interest.

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