Original Article

Antimicrobial Susceptibility Pattern in Diabetic Foot Ulcer: A Pilot Study

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Abstract

Background: Diabetic foot infections (DFIs) are major public health problems and knowledge of microbes that cause infections are helpful to determine proper antibiotic therapy. **Aims:** The aim was to investigate the antimicrobial susceptibility pattern of microbes in DFIs. **Subjects and Methods:** A cross-sectional study was conducted for a period of 6 months at the Department of General Surgery, KMC hospital, Manipal University, Manipal, India. During this period, 108 patients having DFIs admitted in the general surgery wards were tracked from the hospital data management system. These patients' pus samples were examined as Gram-stained smear and cultured aerobically on blood agar and MacConkey agar plates. Antimicrobial susceptibility test was performed by disc diffusion techniques according to Clinical and Laboratory Standards Institute guidelines. **Results:** Of the 108 specimens of the diabetic foot lesions, culture showed polymicrobial growth in 44.4% (48/108). Prevalence of Gram-negative organisms (56%, 84/150) was found to be more than Gram-positive organisms (44%, 66/150). However, *Staphylococcus aureus* was the most frequent pathogen (28%, 42/150). All Gram-positive aerobes were sensitive to doxycycline. All Gram-negative isolates, including extended spectrum beta lactamase producing strains of *Proteus mirabilis* and *Klebsiella oxytoca* except *Acinetobacter* were highly sensitive to amikacin, cefoperazone/sulbactam, and meropenem. *Acinetobacter* was completely resistant to all the common antibiotics tested. **Conclusion:** Prevalence showed Gram-negative bacteria was slightly more than Gram-positive bacteria in diabetic foot ulcers. This study recommends doxycycline should be empirical treatment of choice for Gram-positive isolates and amikacin, cefoperazone/sulbactam, and meropenem should be considered for most of the Gram-negatives aerobes.

Keywords: Antibiotic resistance, Antimicrobial susceptibility, Diabetic foot infection, Diabetic foot ulcer, Multidrug-resistant organism

Introduction

Globally, diabetic foot ulcers are one of the major public health problems leading to socioeconomic burden to the suffering individuals.[1,2] Around 15% of all diabetic patients develop a foot ulcer that is highly vulnerable to infections, at some time in their life.[3] Foot ulcer infections usually spread rapidly on account of polymicrobial growth, predominantly consisting of aerobic, Gram-positive and Gram-negative organisms.[4,5] In recent years, the number of the incidents and complications-related to diabetic foot infections (DFIs) has drastically increased due to increased incidence of multidrug-resistant organisms.[6] Currently, there is a deficit of data on these causatives in diabetic foot, especially in this part of the world. Manipal is located in a region with high literacy, low infant mortality and better access to health care. In this context, data from this region is also likely to be different from data gathered in other parts of rural India.

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Adequate management of these infections needs appropriate antibiotic selection on the basis of culture and susceptibility test reports.[7] Usually initial management often comprises empirical antimicrobial treatment based on local epidemiological data of antimicrobial susceptibility. Knowledge of microbes that cause infections is helpful in determining proper antibiotic therapy.[3] Hence, this pilot study was undertaken in order to investigate

the antimicrobial susceptibility pattern of microbes isolated from diabetic foot ulcers.

Subjects and Methods

A cross-sectional study was conducted in the Department of General Surgery, KMC Hospital, Manipal for a period of 6 months (July 1, 2011–December 31, 2011). During this period, 2892 patients' admission details in the general surgery wards were tracked from the hospital data management system, and identified newly diagnosed 108 diabetic foot ulcer patients. Informed consents were obtained from all the eligible subjects. These patients' pus samples (for bacterial culture) were obtained at the time of admission before starting antibiotic therapy. Wound swabs or pus were collected (deep tissue specimens produce better results than superficial swabs, especially when osteomyelitis is suspected. However, deep tissue specimens are not always practical or available). These specimens were examined as Gram-stained smear and cultured aerobically on blood agar and MacConkey agar plates. Antimicrobial susceptibility test was performed by disc diffusion technique according to Clinical and Laboratory Standards Institute guidelines. Anaerobic culture was not done due to lack of resources for handling anaerobic samples. Hence, results were analyzed for aerobic flora only. Before beginning the study, ethical clearance was obtained from the Institutional Ethical Committee.

Results and Discussion

Baseline characteristics of the 108 diabetic foot ulcer patients taken for the study showed 72.2% (78/108) were males and 27.8% (30/108) were females [Table 1]. Increased male prevalence has been reported in other studies.[3,4] This may be due to higher levels of outdoor activity among males than females. The mean (SD) age of the subjects was 58.3 (7.4) years. The mean (SD) duration of diabetes and hospital stay was 5.6 (4.1) years and 11.6 (7.1) days, respectively. Ulcers were located on plantar digit 33.3% (36/108), plantar metatarsal 22.2% (24/108), dorsal digit 16.7% (18/108), heel 16.7% (18/108), and lateral metatarsal 11.1% (12/108). 22.2% (24/108) patients had a previous history of amputation. Of the 108 specimens from the diabetic foot lesions, culture showed polymicrobial growth in 44.4% (48/108), monomicrobial growth in 44.4% (48/108), and no growth in 11.1% (12/108). Polymicrobial nature of DFIs has been reported from several studies conducted both in this region and abroad.[4] Lipsky, *et al*. have reported the majority of DFIs are polymicrobial nature with aerobic Gram-positive cocci, and especially staphylococci, the most common causative agents.[5] Different result was found by Tiwari *et al*. study, in which monomicrobial infections cases (43.5%, 27/62) were more than polymicrobial infections (35.5%, 22/62).[8]

Organisms isolated from the DFIs are presented in Table 2. Microbiological evaluation of diabetic foot ulcer infections showed that the prevalence of Gram-negative organisms (56%, 84/150) were found to be more than Gram-positive organisms (44%, 66/150) which is in accordance with earlier studies.[1] However, *Staphylococcus aureus* was the most frequent pathogen (28%, 42/150), followed by *Pseudomonas aeruginosa* (24%, 36/150). Almost similar results were obtained by two Indian studies.[3,4] Zubair *et al*. study conducted in North

Table 1: Baseline characteristics of the study group (*n*=108)

Parameters	Values (%)
Mean (SD) age	58.3 (7.4)
Sex	
Male	78 (72.2)
Female	30 (27.8)
Duration of diabetes (years)	5.6±4.1
Duration of hospital stay (days)	11.6±7.1
Lesions involved	
Left foot	66 (61.1)
Right foot	30 (27.8)
Both feet	12 (11.1)
Previous history of amputation	
Yes	24 (22.2)
No	84 (77.8)
Nature of microbial growth	
No growth	12 (11.1)
Monomicrobial	48 (44.4)
Polymicrobial	48 (44.4)
Size of ulcers	
≤4 cm^2	72 (66.7)
>4 cm^2	36 (33.3)
Site of ulcers	
Plantar digit	36 (33.3)
Plantar metatarsal	24 (22.2)
Dorsal digit	18 (16.7)
Heel	18 (16.7)
Lateral metatarsal	12 (11.1)

SD: Standard deviation

Table 2: Bacteria isolated from diabetic foot infections of 96 patients

Bacteria	Number	Percentage
Gram-positive aerobes	66	44
S. aureus (MSSA)	42	28
Enterococcus species	12	8
Beta haemolyic *streptococci*	6	4
S. aureus (MRSA)	6	4
Gram-negative aerobes	84	56
P. aeruginosa	36	24
E. coli	12	8
K. pneumonia	12	8
K. oxytoca (ESBL)	6	4
P. mirabilis (ESBL)	6	4
P. vulgaris	6	4
Acinetobacter	6	4

ESBL: Extended spectrum beta lactamase, MRSA: Methicillin resistant *S. aureus*, MSSA: Methicillin-sensitive *S. aureus, S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. vulgaris: Proteus vulgaris, P. mirabilis: Proteus mirabilis, K. oxytoca: Klebsiella oxytoca*

India also found Gram-negative aerobes were most frequent organisms (63.8%, 65/102) and Gram-positive aerobes (36.1%, 37/102).[9] Al Benwan, *et al*. also reported Gram-negatives were more prevalent, but predominant organisms isolated were members of the *Enterobacteriacea*e.[10] This study revealed that multidrug-resistant (MDR) organisms are very common in hospitalized patients with diabetic foot ulcers. This is in line with the report of Gadepalli, *et al*. study.[3] Zubair *et al*. noticed 45% (46/102) of diabetic foot ulcers were present MDR organisms.[9]

The antimicrobial susceptibility pattern of the Gram-positive and Gram-negative organisms are shown in Table 3. *S. aureus* isolates were 100% (48/48) sensitive to cotrimoxazole and totally resistant to ciprofloxacin. However, some previous studies reported sensitivity to ciprofloxacin.[4] The rate of Methicillin Resistant *S. aureus* (MRSA) in this study was found to be much lower than the studies conducted by Gadepalli, *et al*. and Bansal, *et al*.[3,4] MRSA was resistant to all the antibiotics except linezolid, doxycycline, and cotrimoxazole. Other Gram-positive cocci like *Enterococci* and Beta hemolytic *streptococci* isolates were 100% (12/12, 6/6, respectively) susceptible to chloramphenicol, gentamicin, and doxycycline. All Gram-positive aerobes except *Enterococci* were 100% (54/54) sensitive to cotrimoxazole. Except MRSA, all other Gram-positive aerobes were susceptible to gentamicin. All Gram-positive aerobes were sensitive to doxycycline. Al Benwan *et al*., study showed vancomycin was the most effective antibiotics for Gram-positive bacteria.[10]

Most of studies have reported varying resistance patterns of *P. aeruginosa* toward commonly used antibiotics, but our studies showed a different pattern of susceptibility.[4] Extended spectrum beta lactamase (ESBL) producing *Escherichia coli* was resistant to most of the antibiotics except cefoperazone/sulbactam, meropenem, piperacillin/tazobactam, and ticarcillin/clavulanic acid. Similarly, Gadepalli, *et al*. study also observed ESBL producing *E. coli*.[3] All Gram-negative isolates (but not *Acinetobacter*) including ESBL-producing strains of *Proteus mirabilis* and *Klebsiella oxytoca* were highly sensitive to amikacin, cefoperazone/sulbactam and meropenem. Similarly,

Table 3: Antimicrobial susceptibility (%) pattern of microbes in diabetic foot infections (*n*=96)

Antibiotics	Gram-positive organisms (44%)				Gram-negative organism (56%)						
	S. aureus (MSSA) (28%)	*Enterococcus* spp. (8%)	β hemolytic *streptococci* (4%)	*S. aureus* (MRSA) (4%)	*P. aeruginosa* (24%)	*E. coli* (ESBL) (8%)	*K. pneumonia* (8%)	*P. vulgaris* (4%)	*P. mirabilis* (ESBL) (4%)	*K. oxytoca* (ESBL) (4%)	*Acinetobacter* (4%)
Amikacin	-	-	-	-	66.7	0	100	100	100	100	0
Ampicillin	28.5	0	100	0	-	-	0	0	0	0	0
Augmentin	71.4	100	-	0	-		0	0	0	0	0
Aztreonam	-	-	-	-	100	0	-	100	0	0	0
Cefazolin	66.7	-	-	0	-	0	0	0	0	0	0
Cefuroxime	71.4	-	-	0	-	0	0	0	0	0	0
Cefotaxime	-	-	-	-	-	0	100	100	0	0	0
Ceftazidime	-	-	-	0	100	-	-	-	-	-	-
Cefepime	-	-	-	-	100	0	-	100	0	0	0
Chloramphenicol	-	100	100	-	-	-	-	-	-	-	-
Ciprofloxacin	0	0	100	0	83.3	0	100	100	0	0	0
Clindamycin	71.4	0	-	0	-	-	-	-	-	-	-
Cefoperazone/sulbactam	-	-	-	-	100	100	-	100	100	100	0
Doxycycline	85.7	100	100	100	-	-	-	-	-	--	-
Erythromycin	71.4	0	100	0	-	-	-	-	-	-	-
Gentamicin	83.3	100	100	0	66.7	0	100	0	0	0	0
Linezolid	-	-	-	100	-	-	-	-	-	-	-
Meropenem	-	-	-	-	100	100	-	100	100	100	0
Netilmycin	-	-	-	-	75	0	-	100	0	100	0
Norfloxacin	-	-	-	-	-	-	-	-	-	-	-
Oxacillin	100	-	-	0	-	-	-	-	-	-	-
Penicillin	-	-	100	-	-	-	-	-	-	-	-
Pipercillin	-	-	-	-	100	-	-	-	-	-	-
Pipercillin/tazobactam	-	--	--	-	100	100	100	100	-	0	0
Ticarcillin/clavulanic acid	-	-	-	-	100	100	-	100	0	0	0
TMP/SMX	100	0	100	100		0	0	0	0	100	0
Tobramycin	-	-	-	-	66.7	-	-	-	-	-	-

S. aureus: Staphylococcus aureus, P. aeruginosa: Pseudomonas aeruginosa, E. coli: Escherichia coli, K. pneumonia: Klebsiella pneumonia, P. vulgaris: Proteus vulgaris, P. mirabilis: Proteus mirabilis, K. oxytoca: Klebsiella oxytoca, ESBL: Extended spectrum beta lactamase, MRSA: Methicillin resistant *S. aureus*, MSSA: Methicillin-sensitive *S. aureus*

Al Benwan *et al*. have reported imipenem, piperacillin-tazobactam and amikacin were the most effective antibiotics for Gram-negative bacteria.[10] *Acinetobacter* was totally resistant to all the common antibiotics tested. This was in accordance with multidrug-resistant *Acinetobacter* isolates from Bansal, *et al*. study.[4] The main limitations of our study are a small sample size and the specimen used for the culture that is wound swab and pus.

Conclusion

This study showed most common organisms present in the diabetic foot ulcer were Gram-negative aerobes. However, *S. aureus* was the most predominant organism isolated from the lesions. There were equal proportions of monomicrobial and polymicrobial cultures noticed in the DFIs. 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. Moreover, increasing prevalence of MDR organisms raises serious concerns because MDR infections limit the choice of antibiotic therapy and may lead to poor prognosis. Findings of this study propose that large prospective studies are essential to assess the suitable empirical antibiotic regimen in diabetic foot ulcer infections. This study also directed us toward proper treatment strategies for the management of diabetic foot ulcers with appropriate antibiotics such as doxycycline and gentamicin for Gram-positive cocci and amikacin, cefoperazone/sulbactam and meropenem for Gram-negative bacilli. Clinical guidelines must be implemented to cut the incidence of MDR bacteria in this population and for better patient's outcomes. Simultaneously, we have to seek effective agents for microbes such as MDR *Acinetobacter*.

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