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Original Research Article

Bacteriology of diabetic foot infections and their antibacterial susceptibility

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ABSTRACT

Background: Diabetic foot infections are the most common bacterial infections encountered in patients with diabetes mellitus and remain the leading complication requiring frequent hospitalization. Hence, this study was carried out to determine the prevalence of bacteria in diabetic foot infections and their antibiogram which can help to inform therapeutic choices.
Methods: A prospective study conducted on clinical specimens taken from patients with diabetic foot infections, over 3 years duration. The clinical specimens were processed by using the standard microbiological techniques. The antimicrobial susceptibility pattern was studied by the Kirby-Bauer disc diffusion method.
Results: Among 103 cases studied, 97 were culture positive. Out of these specimens, 25 (25.77%) had monomicrobial flora and 72 (74.23%) had polymicrobial flora. A total of 176 bacteria were obtained which include 62 gram positive cocci and 114 gram negative bacilli. All gram negative bacilli showed good sensitivity to imipenem (97.30%), followed by cefaperazone sulbactam (81.98%), piperacillin-tazobactam (75.68%) and amikacin (72.97%). All gram positive cocci remained 100% sensitive to Vancomycin and Linezolid followed by clindamycin (not tested for Enterococci Spp.) and gentamicin in a range of 91.43 % to 72.88%. The prevalence of multidrug resistant organisms among aerobic isolates were 59.66%.
Conclusions: Diabetic foot infections are polymicrobial. *Pseudomonas aeruginosa* and *Staphylococus aureus* were the most common isolates. The most sensitive antibiotics are imipenem for gram negative bacilli while vancomycin and linezolid were effective for gram positive cocci. The antibiogram of isolates will be helpful in determining the drugs for the empirical treatment of diabetic foot infection.

Keywords: Antimicrobial susceptibility, Bacterial profile, Diabetic foot infections

INTRODUCTION

As the incidence of diabetes in general population is expected to rise, the prevalence of diabetic foot complications will follow.[1] It is estimated that 15% of diabetic patients will develop a foot ulcer during their life time. Infection is a frequent (40%-80%) complication of these ulcers and infection with multidrug resistant organisms (MDRO) are responsible for increased duration of hospitalization, cost of management, morbidity and mortality among diabetic patients.[2-4] Appropriate selection of antibiotics based on the antibiogram of the isolates from the lesions is most

critical for the proper management of these infections. The initial empirical therapy is often decided based on the knowledge of the susceptibility profile of the prevalent microbial flora recovered from the previous cases.

Data about bacteriology and antibacterial susceptibility of diabetic foot infections (DFI) in this region is not available. So, this study was performed to determine the common etiological agents.

METHODS

Study design and sample collection

A prospective study was carried out on patients presented with diabetic foot ulcer at Kamineni Institute of Medical Sciences, Narketpally, Andhra Pradesh, India during a 3-year period. Diabetic foot ulcers were graded according to Wagner classification and ulcers with grade 2 or more, prior to antibiotic therapy were included in the study.[5] Ulcers with Wagner grade 0, 1 and patients on antibiotic therapy at the time of presentation were excluded from the study.

Specimens were collected, after thorough cleaning of the lesion with sterile normal saline. The specimens collected were wound curettage by using a sterile scalpel and aspiration from abscesses by using needle and syringe. The specimens were immediately transported to the microbiology laboratory. Pus swabs were not used as it is not an ideal specimen for isolation of anaerobes.

Study procedure

A Gram stained smear was prepared to look for pus cells and presence of bacteria. The specimens were inoculated onto blood agar and Mac Conkey agar which was incubated over night at 37^0C. All types of colony grown on these plates were identified using standard conventional biochemical methods. An attempt was made to isolate anaerobic bacteria also.

The specimens were collected in thioglycollate broth over layered with liquid paraffin was used for anaerobic culture. The specimen was inoculated on to neomycin blood agar and incubated in anaerobic jar with gaspak chemicals at 37^0C for 48hours. The plates were examined after 48 hours and were reincubated for another 24hours, if no growth was found. Presumptive identification of the isolates from anaerobic culture were done based on the colony morphology, Gram's reaction and cell morphology and results of simple tests like susceptibility to special potency discs like kanamycin 1000µg, colistin 10µg, vancomycin 5µg, Sodium polyanethol sulphonate 100µg, catalase test and spot indole test.[6] A repeat sample was taken from the cases that had isolates of doubtful significance like *Coagulase Negative Staphylococci* (CoNS).

Antibiotic sensitivity testing was done by Kirby Bauer disc diffusion method according to clinical and laboratory standards institute guidelines. Isolates which are resistant to three or more groups of antibiotics considered as MDROs.

RESULTS

The demographic profile of our patients showed that males (73.78%) were more commonly affected than females (26.22%).

Most of the patients (40.78%) belonged to the age group 51-60 years. More than half of the patients had diabetes mellitus for >10 yrs. Majority 93 (90.29%) of the patients had ulcer duration of >1 month (Table 1). Maximum number of patients with diabetic foot ulcer belonged to grades 2 (30.10%) and 3 (41.75%).

Table 1: Duration of diabetes mellitus and duration of diabetic foot ulcers in the patients (n=103).

Duration of diabetes mellitus	Duration of foot ulcers		
	<1 month No (%)	>1 month No (%)	Total No (%)
<5 years	2 (1.94)	12 (11.65)	14 (13.59)
5-10 yrs	5 (4.85)	30 (29.13)	35 (33.98)
>10 years	3 (2.92)	51 (49.51)	54 (52.43)
Total	10 (9.71)	93 (90.29)	103 (100)

Out of 97 culture positive specimens, 25 (25.77%) had monomicrobial flora and 72 (74.23%) had polymicrobial flora. A total of 176 organisms were obtained from the specimens including 170 (96.59%) aerobes and 6 (3.41%) anaerobes. Gram negative bacilli were more (64.77%) than Gram positive cocci (35.23%). There was no growth in specimens from 6 patients. The most common isolates in the present study were *Pseudomonas aeruginosa* and *Staphylococcus aureus* (Table 2).

The antibiotic susceptibility of the aerobic isolates are given in Table 3 and 4. *S. aureus* showed maximum sensitivity to vancomycin and linezolid (100%) followed by clindamycin (75%). MRSA rate in our study was 39.28%. *Enterococc*i were fully sensitive to Vancomycin and linezolid, followed by gentamicin (70.59%). Gram negative isolates were mostly sensitive to Imipenem (97. 30%) followed by cefaperazone sulbactam (81.98), piperacillin tazobactam (75.68), amikacin (72.97), gentamicin (66.67). Antibiotic susceptibility of the 6 anaerobic isolates was not studied.

Out of the 176 organisms, 100 (56.81%) organisms were MDRO (Table 5). These MDROs isolated from 58 patients having history of prior hospital admissions. Prior repeated hospitalization considered to be the risk factor found for development of MDRO. *E. coli* was the most MDRO isolated.

Table 2: Rate of isolation of individual organism from diabetic foot infections (n=176).

Organisms	Number (%)
Gram positive organisms	
Staphylococcus aureus	28 (15.91)
Coagulase negative Staphylococci	14 (7.95)
Enterococcus spp.	17 (9.67)
Peptostreptococcus spp.	3 (1.70)
Gram negative organisms	
Pseudomonas aeruginosa	36 (20.45)
Escherichia coli	18 (10.23)
Klebseilla spp.	16 (9.09)
Citrobacter spp.	13 (7.39)
Proteus spp.	10 (5.68)
Acinetobacter spp.	10 (5.68)
Enterobacter spp.	8 (4.55)
Bacteroid spp.	3 (1.70)
Total	176 (100)

Table 3: *In vitro* activity of antimicrobial agents against Gram positive bacteria (n=59).

Antimicrobial agent	*Staphylococcus aureus* (n=28)	*CoNS* (n=14)	*Enterococci spp.* (n=17)
	Sensitive No:(%)	**Sensitive No: (%)**	**Sensitive No:(%)**
Penicillin	2(7.14)	3 (10.71)	-
Ampicillin	-	-	6(35.29)
Cefoxitin	17(60.72)		
Erythromycin	8 (28.57)	7 (50.00)	-
Cotrimoxazole	15(53.57)	7 (50.00)	-
Ciprofloxacin	12(42.86)	10(71.43)	-
Gentamicin	18(64.28)	13(92.86)	12(70.59)
Clindamycin	21(75)	14(100)	-
Vancomycin	28 (100)	14(100)	17 (100)
Linezolid	28(100)	14(100)	17(100)

Table 4: *In vitro* sensitivity of antimicrobial agents against Gram negative bacteria (n=111).

Agent	*P.aeruginosa* (n= 36)	*E.coli* (n=18)	*Klebseilla spp.*(n=16)	*Citrobacter spp.*(n=13)	*Proteus spp.*(n=10)	*Acinetobacter.spp.* (n=10)	*Enterobacter spp.*(n=8)
	Sensitive No (%)	**Sensitive No (%)**	**Sensitive No (%)**	**Sensitive No (%)**	**Sensitive No (%)**	**Sensitive No (%)**	**Sensitive No (%)**
Amp	-	2(11.11)	0	1(7.69)	3 (30)	0	0
AC	-	4 (22.22)	3(18.75)	2(15.38)	6 (60)	1(10)	1(12.5)
CZ	-	3(16.67)	3 (18.75)	4(30.77)	4(40)	0	0
CXM	-	5 (27.78)	4(25)	4(30.77)	4(40)	0	0
CTX	-	8 (44.44)	6(37.5)	6(46.15)	7 (70)	4(40)	3 (37.5)
CAZ	14 (38.89)	-	-	-	-	-	-
GM	28 (77.78)	12(66.66)	10(62.5)	9(69.23)	6(60)	4(40)	5 (62.50)
AK	30 (83.33)	13 (72.22)	10(62.5)	10(76.92)	6(60)	7 (70)	5 (62.50)
COT	-	10(55.55)	11(68.75)	8(61.54)	7 (70)	3 (30)	4(50)
CIP	22 (61.11)	6(33.33)	5(31.25)	4(30.77)	6 (60)	5(50)	3(37.5)
PIT	25(69.44)	14(77.78)	14(87.5)	11(84.62)	8(80)	6(60)	6(75)
CFS	31 (86.11)	15(83.33)	14(87.5)	11(84.62)	8(80)	6(60)	6(75)
IMI	34 (94.44)	18(100)	16(100)	13 (100)	10 (100)	9 (90)	8 (100)

AC-Amoxycillin Clavulanicacid, CXM- Cefuroxim, CTX- Cefotaxim. CA Z- Ceftazidime, GM-Gentamicin, AK-Amikacin, PIT-Piperacillin tazobactam, CFS-Cefaperazone sulbactam, I-Imipenem, (-) –Not used.

Table 5: Prevalence of multidrug resistant organisms (MDRO) in diabetic foot infection.

Organisms	Total number	MDRO (No%)
Staphylococcus aureus	28	14(50.00%)
Coagulase -ve Staphylococci	14	6(42.86%)
Enterococci spp.	17	11(64.71%)
Pseudomonas aeruginosa	36	16(44.44%)
Escherichia coli	18	17(94.44)
Klebseilla spp.	16	11(68.75%)
Citrobacter spp.	13	7(53.85%)
Proteus spp.	10	7(70%)
Acinetobacter spp.	10	6(60)
Enterobacter spp.	8	5(62.5)
Total	176	100(56.81%)

DISCUSSION

Diabetic foot ulcer is one of the most common complication requiring hospitalization among diabetic patients.[7] The mean age of diabetic foot ulcer cases study in us was 56.21±10.15 years. This is in correlation with the study of Gadepally et al and Ekta et al.[8,9] Male predominance was seen in our study which is same as the studies done by Viswanathan et al and Umadevi et al.[10,4] This could be due to more outdoor activities in males, having more chances of getting trivial injuries leading to chronic non-healing ulcers. Approximately half of the patients (52.43%) in the present study were having diabetes for more than 10 years of duration which is in correlation with the study conducted by Ekta et al and

Gadepally et al.[9,8] Diabetes mellitus for more than 10 years duration is a risk factor for development of foot ulcers.[11] Most of the patients in the present study had ulcer for more than one month duration which is in correlation with the study conducted by Sharma et al.[12] More the duration of foot ulcers, more the risk of getting infections with polymicrobial flora.[9] Average number of organisms per case in our study was 1.7 which is in correlation with Umadevi et al.[4] The rate of isolation of the polymicrobial flora (74.23%) in the present study is correlating with Anandi et al.[13] The interaction of the organisms within the polymicrobial mixture leads to the production of virulence factors such as hemolysins, proteases and collagenases as well as short chain fatty acids that causes inflammation, impede wound healing, and contribute to the chronicity of infection. In such mixtures, biofilm that impede the penetration of antimicrobial agent into infected site may also form.[14]

The prevalence of the aerobic organisms was more than the anerobic organisms in our study, which is similar to the studies conducted by Ahmed et al and Abdulrazak et al.[15,16] However, very low prevalence of the anaerobic organisms in the present study is due to the constrained resources for identification of the anaerobic isolates which is a drawback of our study. Gram negative bacteria (64.77%) were more prevalent than Gram positive bacteria (35.23%) in our study which is in correlation with studies of Ekta et al and Gadepally et al.[9,8] The most common isolates in our study are *P. aeruginosa* and *S. aureus* that is consistent to the previous studies.[15,9]

Among Gram positive isolates *S. aureus*, *CoNS*, *Enterococci* were 100% sensitive to vancomycin and linezolid. Seventy-five percentage of *S. aureus* isolates were sensitive to clindamycin. The MRSA rate among the *S. aureus* isolates was 39.28%. In our study, Gram negative isolates were mostly sensitive to imipenem (97. 30%). The other more sensitive drugs for Gram negative bacilli were cefaperazone sulbactam (81.98), piperacillin tazobactam (75.68), amikacin (72.97), gentamicin (66.67). Aminoglycoside sensitivity in our study is similar with the study of Chincholikar et al.[17] The MDRO were isolated from 58 patients with diabetic foot ulcer having history of previous hospital admissions. The prevalence of MDRO in the present study is lesser than the study conducted by Sasikala et al.[18] Infection with MDRO of diabetic foot leads to increased risk of amputation and fatality rate.

CONCLUSION

Diabetic foot infections are polymicrobial with a predominance of Gram negative bacteria. Infections with MDRO are common especially in patients with previous hospital admissions. Prompt initiation of appropriate antibiotic therapy, as well as surgical debridement of necrotic or de vascularized soft tissue is essential for controlling the infection. Life threatening infection can be treated with imipenem and vancomycin. Appropriate usage of antibiotics for diabetic foot infection based on local antibiogram pattern can certainly help the clinician in reducing the burden of diabetic foot complications like amputations.

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