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Bacteriology of Diabetic Foot Ulcers

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A prospective study was carried out on patients with diabetic foot ulcers to determine their bacteriological spectrum and to assess their in vitro susceptibility to the commonly used antibiotics. The criterion for inclusion was a diabetic patient with foot ulcer of grade 1 or more according to Meggit Wagner Classification System. Polymicrobial growth was seen where 236 bacteria were isolated with an average of 2.36 per case. Of these isolates, 218 (92.37%) were aerobes and 18(7.63%) were anaerobes. Staphylococcus aureus(22.01%) was the predominant aerobe isolated followed by Proteus mirabilis(19.7%). 16.7% of Staphylococcus aureus were methicillin resistant. All gram positive aerobes showed 100% sensitivity to vancomycin. All gram negative aerobes except Klebsiella spp. and Pseudomonas aeruginosa showed 100% sensitivity to amikacin. The major anaerobes isolated were Bacteroides spp(61.1%) followed by Peptostreptococcus spp(27.8%) and Clostridium spp.(11.1%).

Key words: Diabetic foot ulcer, Polymicrobial, Wagner's grades.

Diabetes mellitus is a chronic disorder affecting a large segment of population and also a major public health problem.[1] Diabetes is rightly called a disease of complications and iceberg disease. India homes 33 million diabetics, ranking highest in the world and has a prevalence of about 8% in urban India. 20% of all diabetic complications involve feet.[2] The trio of problems leading on to the diabetic foot is neuropathy, vascular changes and infections which constitute diabetic foot syndrome.[3,4]

The burden of diabetic foot is set to rise further in future since its contributory factors such as peripheral neuropathy and peripheral vascular disease are present in >10% of the cases at the time of diagnosis.[5] The infection leads to the early development of complications even after a trivial trauma, the disease progresses and becomes refractory to antibacterial therapy[6]. Selecting appropriate antimicrobial therapy requires a knowledge of likely etiologic agents. About 10-30% of diabetic patients with a foot ulcer will eventually progress to an amputation, which may be minor (foot sparing) or major. Conversely, an infected foot ulcer precedes approximately 60% of amputations, making infections perhaps the most important proximate cause of this tragic outcome[7]. It is essential to assess the magnitude of bacterial infection of the lesions to avoid further complications and save the diabetic foot. Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy and to avoid further complications.[1]

In view of the above, a prospective bacteriologic study was undertaken to assess the role of aerobic and anaerobic bacteria in diabetic foot ulcers. The antimicrobial spectrum of these isolates would assist the clinicians in the therapy of this dreaded complication of diabetes.

MATERIALS AND METHODS

Study period

March 2006- February 2007

Study Setting

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This study was approved by the Institutional Ethical Committee. A total of 100 diabetic patients with foot ulcer from Surgery Unit of Victoria Hospital were included in this Hospital based cross-sectional study. The foot ulcers were categorized into 6 grades (grade 0- grade 5) based on Meggit Wagner Classification System.[8] The criterion for inclusion was a diabetic patient with foot ulcer of grade 1 or more. The ulcer was washed with normal saline, superficial dead tissue and slough was removed with sterile scissors and scalpel. Three debrided tissue samples were taken from each patient and were subjected for smear preparation, aerobic and anaerobic culture. The smears were prepared on the bedside by crushing the tissue sample between two sterile glass slides and heat fixed. The tissue sample for aerobic culture was inoculated into Brain Heart Infusion (BHI) broth and the tissue sample for anaerobic culture was inoculated into Robertson's Cooked Meat broth (RCMB) at bedside.

In the Department of Microbiology, the smears were Gram stained. BHI broth was subcultured onto 5% sheep blood agar, Mac conkey agar and chocolate agar plates. The growth was identified according to the standard microbiological procedures[9]. Antibiotic susceptibility testing was performed by the Kirby Bauer disc diffusion method as recommended by the Clinical Laboratory Standards Institute[10]

The inoculated RCMB was subcultured onto 1% neomycin blood agar plate and a gentamicin disc was placed at the junction of primary and first streaking while a metronidazole disc was placed at the junction of first and second streak lines to presumptively identify anaerobes.[11] The neomycin blood agar plates were incubated anaerobically at 37°c in an anaerobic jar(Hi Media Anaerobic System Mark II LE 002 3.5L) with Gaspak(Hi Media).The bacteria were identified using gram staining and colony morphology. Due to lack of resources, further identification of anaerobic cultures was not done.

Data was collected and analysed by descriptive statistics. Continuous variables were expressed as mean with standard deviation while categorical variables were expressed as percentages.

RESULTS

Among 100 diabetic foot ulcer patients studied, 65(65%) were males and 35(35%) were females with male-to-female ratio being 1.85:1. The age ranged from 25 to 75 years (average= 53.79 years; s.d= 9.8 years). A total of 236 bacteria were isolated from these patients which represent an average of 2.36 bacteria per case. The bacteria isolated are summarized in Table 1. Among aerobes, Gram negatives accounted for 140(64.2%) and Gram positives comprised 78(35.8%). The ratio of Gram negative to Gram positive aerobic bacteria was 1.79:1. Staphylococcus aureus was the most common isolate accounting for 48(22.01%) followed by Proteus mirabilis 22(19.7%) and others as shown in Table 2. Among anaerobes, Bacteroides spp 11(61.1%) was most commonly isolated followed by others as shown in Table 3.

Table 1. Distribution of aerobes and anaerobes

Bacteria	No. of bacteria	Percentage
Aerobes	218	92.37
Anaerobes	18	7.63
Total	236	100

Table 2. Frequency of aerobes

Aerobes	No.of bacteria	Percentage
Staphylococcus aureus	48	22.01
Proteus mirabilis	43	19.7
Pseudomonas aeruginosa	22	10.0
Klebsiella pneumoniae	21	9.6
Escherichia coli	21	9.6
Enterococcus faecalis	12	5.5
Coagulase Negative Staphylococcus	11	5.04
Proteus vulgaris	9	4.12
Klebsiella oxytoca	8	3.67
Citrobacter freundii	7	3.21
Acinetobacter spp	6	2.75
Corynebacterium spp	5	2.2
Enterobacter spp	3	1.37
Group A *Streptococci*	2	0.9
Total	218	100

Antibacterial susceptibility testing revealed that *Staphylococcus aureus* showed 81.2% susceptibility to cefotaxime, 83.3% to oxacillin. 77.1%, 62.5%, 66.7% were resistant to penicillin, erythromycin and ampicillin respectively. Enterococcus faecalis showed 66.6% sensitivity to ampicillin. Coagulase negative *staphylococcus* showed 90.9% sensitivity to ciprofloxacin. All gram positive aerobes showed 100% sensitivity to vancomycin as shown in Table 4. Proteus mirabilis was 100% sensitive to amikacin, 95.34% sensitive to ceftriaxone and ceftazidime and 93.02% to cefotaxime. Highest sensitivity among

Table 3. Frequency of anaerobes

Anaerobes	Number	Percentage
Bacteroides spp	11	61.1
Peptostreptococcus spp	5	27.8
Clostridium spp	2	11.1

Table 4. Antibiotic susceptibility pattern of gram positive aerobes

Aerobes	P	E	A	Ac	G	Ak	Cf	Co	Cp	Ox	V
Staphylococcus	11	18	16	30	26	33	22	30	28	40	48
aureus	(22.9)	(37.5)	(33.3)	(62.5)	(54.1))	(68.7)	(45.8)	(62.5)	(58.3)	(83.3)	(100)
Enterococcus	3	6	8	-	3	6	6	3	5	-	12
faecalis	(25)	(50)	66.6)		(25)	(50)	(50)	(25)	(41.6)		(100)
CONS	5	7	5	9	6	10	10	8	5	11	11
	(45.4)	63.6)	(45.4)	(81.8)	(54.5)	(90.9)	(90.9)	(72)	(45.4)	(100)	(100)

Figures shown in table are number of sensitive isolates
Figures shown in parenthesis indicate percentage of sensitive isolates
P-Penicillin, E-Erythromycin, A-Ampicillin, Ac-Amoxiclav, G-Gentamicin, Ak-Amikacin, Cf-Ciprofloxacin, Co- Cotrimoxazole, Cp-Cephalexin, Ox-Oxacillin, V-Vancomycin

Table 5. Antibiotic susceptibility pattern of gram negative aerobes

Aerobes	A	G	Ak	Nt	Cb	Cf	Co	Cp	Cu	Ce	Ci	Ca
Proteus	12	14	43	-	-	36	30	36	38	40	41	41
mirabilis	(27.9)	(32.5)	(100)			(83.7)	(69.7)	(83.7)	(88.3)	(93.0)	(95.3)	(95.3)
Pseudomonas	-	12	19	15	17	18	12	10	10	16	18	21
aeruginosa		(54.5)	(86.3)	(68.1)	(77.2)	(81.8)	(54.5)	(45.4)	(45.4)	(72.7)	(81.8)	(95.4)
Klebsiella	2	7	18	-	-	10	10	4	9	16	18	19
pneumoniae	(9.5)	(33.3)	(85.7)			(47.6)	(47.6)	(19.0)	(42.8)	(76.1)	(85.7)	(90.4)
Escherichia	17	19	21	-	-	18	12	15	18	19	20	19
coli	(80.9)	(90.4)	(100)			(85.7)	(57.1)	(71.4)	(85.7)	(90.4)	(95.2)	(90.4)
Proteus	3	7	9	-	-	9	6	8	7	7	9	9
vulgaris	(33.3)	(77.8)	(100)			(100)	(66.6)	(88.8)	(77.8)	(77.8)	(100)	(100)
Klebsiella	1	2	6	-	-	3	4	1	3	4	7	7
oxytoca	(12.5)	(25)	(75)			(37.5)	(50)	(12.5)	(37.5)	(50)	(87.5)	(87.5)
Citrobacter	4	5	7	-	-	5	4	4	6	6	6	6
freundii	(57.1)	(71.4)	(100)			(71.4)	(57.1)	(57.1)	(85.7)	(85.7)	(85.7)	(85.7)
Acinetobacter	1	4	6	-	-	5	3	1	3	5	6	6
spp	(16.6)	(66.7)	(100)			(83.3)	(50)	(16.6)	(50)	(83.3)	(100)	(100)
Enterobacter	0	2	3	-	-	3	1	1	2	3	3	3
spp		(66.7)	(100)			(100)	(33.3)	(33.3)	(66.7)	(100)	(100)	(100)

Figures shown in table are number of sensitive isolates
Figures shown in parenthesis indicate percentage of sensitive isolates
A-Ampicillin, G-Gentamicin, Ak-Amikacin, Nt- Netillin, Cb- Carbenicillin, Cf-Ciprofloxacin, Co- Cotrimoxazole, Cp-Cephalexin, Cu- Cefuroxime, Ce- Cefotaxime, Ci-Ceftriaxone, Ca-Ceftazidime

Pseudomonas aeruginosa was seen to ceftazidime (95.4%). All gram negatives showed 100% sensitivity to amikacin except *Pseudomonas aeruginosa* and *Klebsiella* spp. as shown in Table 5.

DISCUSSION

In the present study, a total of 236 bacteria were isolated from 100 diabetic foot ulcer patients with an average of 2.36 bacteria/case. Polymicrobial nature of diabetic foot infections has been observed in various studies.[1,12,13,14] Among 236 bacteria, 218(92.37%) were aerobes and 18(7.63%) were anaerobes. 85% and 84.7% aerobic isolation has been reported by Vijaya etal and Ravisekar etal.[15,16] The same authors have reported 15% and 15.3% anaerobic isolation respectively. Most of our patients had Grade 2 ulcers which are usually uncomplicated. This may be the reason for our low isolation of anaerobes and high isolation of aerobes. In our study, *Staphylococcus aureus*(22.01%) was the predominant aerobic isolate and Bacteroides spp(61.1%) was the predominant anaerobic isolate. These findings are in concordance with Wheat etal, Ramani etal, Dipali AC *et al.,* and Vijaya *et al.,* [17,13,18,15] who isolated Staphylococcus aureus as their major aerobe, 37%, 27.7%, 31.25%, 32.94% respectively. These authors except Wheat *et al.,* also isolated Bacteroides spp as their major anaerobe, 39.34%, 42.2% and 40% respectively.

As regards the antibiotic sensitivity pattern of Gram positive aerobes, *Staphylococcus aureus* isolates were uniformly susceptible to vancomycin with more than 50% sensitivity to cephalosporins. 16.7% of them were MRSA. This is in concordance with the findings by Anandi etal[1] and Shankar etal[19] where MRSA were 20.8% and 10.3% respectively. All other gram positives were 100% sensitive to vancomycin.

Among gram negative aerobes, Proteus mirabilis which was the preominant isolate showed 100% sensitivity to amikacin with 95.34% sensitivity to ceftriaxone and ceftazidime each. Sensitivity to cefuroxime and cefotaxime was 88.3% and 93.02% respectively. This could be explained by the fact that Proteus spp are known to produce unique beta lactamase (cefuroximase) that has high activity mainly against cefuroxime and cefotaxime[20]. All gram negative aerobes showed 100% sensitivity to amikacin except Pseudomonas aeruginosa, Klebsiella pneumoniae and Klebsiella oxytoca where sensitivity to amikacin was 86.3%, 85.71% and 75% respectively. Anandi etal observed that all aerobes were sensitive to amikacin except 2 Pseudomonas spp isolates. Dipali AC etal found that 95.74% of the aerobic gram negative bacilli were sensitive to amikacin.

The limitation of this study was the failure to study the production of Extended Spectrum β Lactamase, Amp C β-Lactamase and Metallo β-lactamase.

CONCLUSION

Diabetic foot ulcers often harbour polymicrobial infections. MRSA can complicate the already deteriorating ulcer and increase the cost of therapy. A combination regimen consisting of amikacin and vancomycin seems to be the most prudent empirical treatment of diabetic foot infection for aerobes.

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