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Case Series

Bacteriological profile of diabetic foot

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ABSTRACT

Foot infections in patients with diabetes mellitus are a major cause of morbidity, can lead to gangrene and ultimately amputation of the limb. Aim of the study was to determine the type of microorganisms isolated from the Diabetic foot ulcer and antibiotic resistance pattern. We have included 50 patients having diabetic foot ulcers of Wagner's grade 1 or above in our study. Debrided tissue, pus, or swabs from the base of the ulcers were subjected to aerobic and anaerobic culture. The organisms were identified, and further antibiotic sensitivity was conducted. Seventy-two aerobic and 13 anaerobic organisms were isolated. Among the aerobic and anaerobic bacteria isolated, most predominant organisms were *S. aureus* and *Bacteroides* spp.; respectively. Of the *S. aureus*, 77.8% were methicillin resistant, while 42.1% of gram-negative *Enterobacteriaceae* were extended spectrum beta-lactamase (ESBL) positive. *Klebsiella* spp. was the highest ESBL producer. *Acinetobacter* spp. was the highest MBL producer. Most sensitive drugs for *S. aureus* were linezolid, vancomycin and amikacin. Gram-negative bacteria were mostly sensitive to piperacillin-tazobactam and amikacin. *Pseudomonas* spp. were usually sensitive to meropenem, piperacillin - tazobactam. *Acinetobacter* spp. was sensitive to colistin, tigecycline. As diabetic ulcers are often infected by multidrug-resistant bacteria, a knowledge of the common bacterial pathogens implicated as well as their sensitivity pattern helps the clinician to choose the proper antibiotic for a timely treatment.

Keywords: Diabetic foot infection, Amoxicillin-clavulanic acid, Cefotaxime, Ceftriaxone, Ceftriaxone-sulbactam, Amikacin, Gentamycin, Linezolid, Azithromycin, Piperacillin-tazobactam

INTRODUCTION

Foot wounds are an increasingly common problem in people with diabetes and now constitute the most frequent diabetes-related cause of hospitalization.[1] People with diabetes have about a 12-25% chance of developing a foot ulcer in their lifetime, thus contributing to a major public health issue.[2,3] Rate of amputation of a limb is estimated to be forty times greater in infected non-healing ulcer in diabetics than the patients in trauma.[4,5] Amputation is even more likely when DFI and foot ischemia coexist.[6]

The pathophysiology of foot infections in persons with diabetes is quite complex, but their prevalence and severity are largely a consequence of host-related disturbances (immunopathy, neuropathy and arteriopathy) and secondarily pathogen-related factors (virulence, antibiotic-resistance and microbial load).[7,8]

Because many different organisms, alone or in combination, can cause a DFI, selecting the most appropriate antibiotic therapy requires defining the specific causative pathogens.[8-10] Clinicians should avoid antibiotic therapy that is unnecessary, overly broad-spectrum or excessively prolonged, as it may cause drug-

related adverse effects, incurs financial cost and encourages antimicrobial resistance.[10]

Foot problems are largely preventable, and successful treatment depends on the correct evaluation of the patient, diagnosis, and proper management of infection.

CASE SERIES

A total 50 diabetic patients with ulcers of Wagner's grade 1 or above during the period of May-Oct 2019 in general surgery ward, at Dr. D.Y. Patil hospital and research center, Pune who consented were enrolled in this study. Local examination of the foot and ulcer was done, grading was carried out as per Wagner's system.

Biochemical, hematological, serological and radiological profiles of the patients were noted. Ulcer surfaces were rinsed with sterile normal saline, and swabs were collected from the base of the ulcer after debriding the superficial exudates. Pus if present was aspirated using a sterile syringe. The debrided necrotic tissue, pus or swab was put into preheated Robertson's cooked meat medium and incubated anaerobically in McIntosh-Fildes jar at 37°C and then sub cultured in anaerobic condition on neomycin blood agar plate.

For aerobic culture, the samples were inoculated on pre dried plates of blood agar and MacConkey agar along with a nutrient broth. The colonies grown on the plates after overnight incubation at 37°C were identified. Anaerobes isolated were identified to the genus level only. Antimicrobial susceptibility testing of aerobic isolates was done using Kirby-Bauer's disc diffusion method on Muller-Hinton agar plates.

Outcomes

In this study of total 50 diabetic patients presenting with ulcers, 39 (78%) were males and 11 (22%) were females. Most of the patients (54%) belonged to the age group of 51-60 years. Apart from one case of type 1 diabetes mellitus, all others were of type 2 variety. Majority of the cases presented with ulcer within 6-10 years of being diagnosed as a diabetic, whereas 8.2% of cases presented even after 20 years of diagnosis. Most of the patients came to this hospital only after 2-3 weeks of the development of foot ulcers. Half of these patients were having a combination therapy of insulin and oral hypoglycemic agents. However, 17.6% of cases had either left treatment or were irregularly taking the medications leading to an uncontrolled blood sugar.

Of the 50 cases, 29 (58%) were monomicrobial, 17 (34.0%) were polymicrobial, and only 4 (08%) cases were sterile in culture. About 25% of grade 1 ulcers did not reveal any growth of organisms, the percentage of which is drastically reduced as the grade of ulcer increased. Percentage of ulcers with poly-microbial etiology increased as the grade of ulcer increased such that 50% of gangrenous ulcers were polymicrobial (Table 1).

Table 1: Distribution of organisms isolated according to grades.

Wagner's grade of ulcer	Number of organisms isolated (percentage of total ulcers)		
	Organism 0	Organism 1	2 or more organisms
Grade I	1 (25)	3 (75)	0 (0)
Grade II	2 (11.11)	12 (66.66)	4 (22.22)
Grade III	1 (5.55)	11 (61.11)	6 (33.33)
Grade IV	0 (0)	3 (37.5)	5 (62.5)
Grade V	0 (0)	0 (0)	2 (100)

As per Wagner's grading system, grade 2 and 3 ulcers were most predominant (72%) in this study followed by grade 4 and 5 ulcers (20%). Out of 50 ulcers, total no. of aerobes and anaerobes isolated were 72 (84.7%) and 13 (15.29%) resp. Number of organisms per ulcer in this study were 1.7. No. of aerobic and anaerobic organisms isolated per sample increased with the rise in grade of ulcer. Anaerobic organism isolation was highest in grades 4 and 5 ulcers. Only aerobic bacteria were isolated in 37 ulcers while in 13 ulcers anaerobic organisms were present in addition to aerobic microbes (Table 2).

Table 2: Variation of number of organisms isolated based on grades.

Grade (no. of ulcer)	G I (4)	G II (18)	G III (18)	G IV (8)	G V (2)
Aerobes	2	18	23	25	4
Anaerobes	1	2	3	5	2
Aerobes/ sample	0.5	l	1.27	3.125	2
Anaerobes/ sample	0.25	0.11	0.16	0.27	1
Organisms/ sample	0.75	l.11	1.43	3.395	3

Table 3: Different types of organisms isolated from the diabetic ulcers.

Organisms	Percentage (%)
Aerobes	
Staphylococcus aureus	18 (25)
CoNS	01(1.38)
***Enterococcus* spp.**	01 (1.38)
S. Pyogenes	01 (1.38)
E. Coli	07 (13.88)
K. Pneumoniae	13 (18)
Enterobacter spp.	03 (4.16)
Citrobacter Freundii	04 (5.55)
Proteus mirabilis	03 (4.16)
Pseudomonas Aeruginosa	17(23.6)
***Acinetobacter* spp.**	04 (5.55)
Anaerobes	
***Bacteroides* spp.**	08 (61.5)
***Peptostreptococcus* spp.**	05 (38.5)

Table 4: Sensitivity pattern of various organisms.

Organism	AMC	CTX	CTR	CFS	AK	GEN	LZ	AZM	Ciprofloxacin	Colistin	Tigecycline	Clindamycin	Erythromycin	Vancomycin	Chloramphenicol	Meropenem	PIT
Staphylococcus aureus	27.6	15	12.8	61	74	72.2	96	27.6	14	-	-	-	-	95.6	-	-	70
CONS	0	0	0	100	100	100	-	-	-	-	-	-	-	100	-	-	100
***Enterococcus* spp.**	100	100	100	100	100	100	-	-	-	-	-	-	-	100	-	100	100
E. coli	10	14	12	24	90	88	-	-	-	-	-	-	-	-	-	100	100
K. pneumoniae	0	10	18	28	76	50	-	-	-	-	-	-	-	-	-	100	80
***Enterobacter* spp.**	0	0	0	0	0	0	0	0	0	100	100	-	-	-	-	-	-
Citrobacter freundii	20	16	18	20	74	72	-	-	-	-	-	-	-	-	-	100	100
Proteus mirabilis	18	25	25	40	80	80	-	-	-	-	-	-	-	-	-	100	100
Pseudomonas aeruginosa	0	0	5	50	80	80	-	-	75	-	-	75	-	-	-	90	90
***Acinetobacter* spp.**	0	0	0	0	0	0	-	-	-	100	100	-	-	-	-	0	0

*AMC-amoxicillin- clavulanic acid, CTX- cefotaxime, CTR-ceftriaxone, CFS-Ceftriaxone-Sulbactam, AK-amikacin, GEN-gentamycin, LZ-linezolid, AZM-azithromycin, PIT-piperacillin-tazobactam.

Among the pathogens isolated by aerobic culture, most predominant organism was *Staphylococcus aureus* (25%), followed by *Pseudomonas aeruginosa* and *Escherichia coli* as shown in the Table 3.

Most of the *Staphylococcus aureus* were sensitive to linezolid (96%), vancomycin (95%) and amikacin (74%). Among the aminoglycosides, amikacin was the most sensitive drug (74%). They showed 74% and 70% sensitivity to gentamycin and piperacillin-tazobactam respectively. Nearly 77.8% of *S. aureus* were methicillin-resistant *S. aureus* (MRSA). Coagulase-negative *Staphylococcus* was more susceptible to the antibiotics than *Staphylococcus. aureus* and showed sensitivity to all the antibiotics. *Enterococcus* spp. isolated was sensitive to most of the antibiotics.

In the present study, tigecycline and colistin were the most useful antibiotics for the treatment of isolates for members of *Enterobacteriaceae* family. These isolates were mostly sensitive to piperacillin-tazobactam and amikacin. Amoxicillin-clavulanic acid and cephalosporins were the most resistant antibiotic.

Non fermenters *(Pseudomonas* spp. and *Acinetobacter* spp.) showed a higher degree of resistance to imipenem than those of *Enterobacteriaceae*. *Pseudomonas* spp. were usually sensitive to meropenem (90%), piperacillin-tazobactam (90%), amikacin (80%), gentamycin (80%) and whereas *Acinetobacter* spp. was mostly sensitive to colistin (100%), tigecycline (100%).

Highest degree of production of ESBL and MBL was shown by *Klebsiella* spp. and *Acinetobacter* spp., respectively (Table 4).

DISCUSSION

In this study, most of the patients (54%) belonged to the age group of 51-60 years; the males were more than females and patients mostly developed ulcers within 6-10 years of the detection of diabetes mellitus which is comparable to the studies done by Prompers and Criado et al.[11,12] It has also been stated that male diabetic patients with multidrug-resistant Gram-negative bacilli-infected foot ulcers have poor glycemic control and have higher mortality than their female counterparts.[13] An alarming fact was that 5.4% of the included patients were diagnosed as type 2 diabetics only after having a foot ulcer.

In our study, 58% of wound cultures showed monomicrobial flora and 16% had polymicrobial flora. This is similar to studies done by Zubair and Raja et al.[14,15] The higher incidence of monomicrobial flora in this study than studies done by Chincholikar and Amalia et al is probably due to the higher prevalence of mild and superficial ulcers.[16,17] Almost 10% of ulcers had no bacterial growth which may be due to the prior treatment with broad-spectrum antibiotics in most of the cases (94.6%).

In our study, the number of organisms per ulcer is 1.7. which correlates with studies done by Zubair and Raja et al.[14,15] However, other studies show higher number of isolates per ulcer.[18-20]

The number of organisms per ulcer varies significantly with the grade of ulcer, time of presentation after development of ulcer, as well as the sampling techniques. Prior antibiotic therapy may have also influenced the outcome in our study.

Isolation of anaerobic bacteria (15.29%) is lesser than other studies in which it can be up to 51.56%.[9,15,20] Rate of isolation of anaerobes varies as per the method of sampling, prior antibiotic therapy, and type of wound. It has been postulated that, in the superficial grades (Wagner 1 and 2), aerobic bacteria *(Staphylococcus* spp., *Streptococcus* spp., and *Enterobacteriaceae)* are the predominant pathogens, while anaerobic bacteria add up in Wagner's grade 3-5 ulcers.[21] In our study, most of the anaerobes were isolated from grade 4 to grade 5 ulcer cases. In this study, most common anaerobic isolates were *Bacteroides* followed by *Peptostreptococcus.* This correlates with studies done by Sapico and Bamberger et al where *Bacteroides* spp. have been postulated as the most prevalent anaerobe associated with diabetic ulcers.[19,22]

Gram-positive aerobic bacteria were more frequently isolated in comparison to Gram negatives which correlates with studies done by Fejfarova and Dang et al but few other studies done by Umadevi et al.[23-25] and Mohanasoundaram et al show gram negative isolates as the most predominant aerobic infection in diabetic foot ulcers.[26]

Sensitivity pattern of the microbes in diabetic foot ulcers is often heralded by the presence of multidrug-resistant strains. The presence of MDR organisms is the only significant independent predictor of glycemic control.[20] About 77.8% of *S. aureus* isolated in the present study were methicillin resistant which may vary from 10.6 to 71.4% in various studies.[9,20,27] Seven strains of vancomycin-resistant *S. aureus* were found in the present study. Similarly, ESBL production was noted in 42.1% of gram-negative isolates. *Klebsiella* spp. was the highest ESBL producer. *Acinetobacter* spp. was the highest MBL producer. *Citrobacter* spp., although had a low rate of ESBL production (46.1%) was highly resistant (83.4%) to cephalosporins, thus indicating other mechanisms of resistance. The MBL enzymes which hydrolyze all beta-lactam drugs and carbapenems were commonly associated with *Acinetobacter* spp. Association of MBL-producing strains in diabetic ulcers can lead to the high incidence of treatment failure.

CONCLUSION

Diabetic ulcers are frequently treated with empiric antibiotics. The antimicrobial susceptibility pattern and severity of wound often determine the choice of empiric treatment. In our study, *S. aureus* was the most predominant bacterial cause. The type and number of infecting organisms vary as per the grade of ulcer. In grades 4 and 5 ulcers, it is important to include the treatment for anaerobic organisms as well. There is an increasing percentage of multidrug resistance organisms associated with these ulcers which dims the prognosis. Linezolid can be used for empiric therapy for lower grade ulcers while meropenem, linezolid, and metronidazole can be used for higher grade ulcers. Nevertheless, proper knowledge of foot care is essential to prevent ulceration as well as for the early diagnosis of diabetic foot.

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