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Bacteriological Evaluation of Diabetic Foot Ulcers

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ABSTRACT

Diabetes mellitus is the major metabolic disorder which is one of the most common causes of morbidity and mortality in India. Diabetic foot ulcer, the challenging clinical complications of diabetes mellitus is usually ignored by diabetic patient. The objectives of this study were to analyse the spectrum of bacteria in diabetic foot infections (DFIs) and to study their antibiotic susceptibility pattern. The current study was conducted on 50 diabetic male and female patients with foot ulcers, at tertiary care centre in Chennai, from October 2012 to February 2013. Of the 50 patients studied, 52 organisms were isolated which represent an average of 1.04 organisms per case. Aerobic bacteria accounted for 94% of the total isolates. *Escherichia coli* and *Pseudomonas aeruginosa* were the most frequent Gram negative pathogens and *Staphylococcus aureus* was the most common among Gram positive organisms. Antibiotic susceptibility of *S. aureus* revealed that 36.4% were Methicillin resistant *S. aureus* (MRSA) and all were susceptible to vancomycin. Gram negative bacilli were susceptible to ceftriaxone, ceftazidime, ciprofloxacin and aztreonam. DFIs are a real public health concern. Diabetic patient with foot complications are highly vulnerable to amputation of foot or leg. Combination of antimicrobial agents would be more appropriate for empiric treatment. The choice of antimicrobials should be based on the severity of infection, the organisms isolated and sensitivity pattern.

Keywords: diabetic foot ulcer, diabetic foot infections, antimicrobial resistance

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INTRODUCTION

Diabetes mellitus is one of the common diseases in Indian population. Diabetic foot ulcer, the challenging clinical complications of diabetes mellitus are usually ignored by diabetic patient and it is often threatening and disabling. The peripheral diabetic neuropathy, vascular insufficiency and altered functions of leucocytes lead to foot ulcers following trivial injury or trauma [1].

Bacterial infections of diabetic foot ulcers interfere with healing and progress to chronic non- healing wounds. It is the major cause for frequent hospitalization and can lead to the dreadful consequence of lower extremity amputations. Foot infections can result in mental and socioeconomic instability. To avoid the diabetic foot complications, it is foremost necessity to assess the spectrum of virulent bacterial pathogen of the lesion and medical intervention at the earliest.

The aetiology of diabetic foot infection (DFI) is polymicrobial in character. *Staphylococcus aureus* and *Streptococcus* species (spp.) [2, 3] are the common aerobic Gram positive organisms isolated from the wound. The Gram negative aerobic pathogens of infected ulcers include *Proteus* spp., *Escherichia coli* and other spp. of *Enterobacteriaceae [*2, 3]. *Peptostreptococcus* spp., *Bacteroides melaninogenicus* and *Bacteroides fragilis* are the mostly cultured anaerobic organisms [4]. Indiscriminate antimicrobial usage results in the emergence of antibiotic resistance against the common antibiotics.

The optimized antibacterial regimen should be established based on the isolation of the causative organisms and its sensitivity pattern. The spectrum of bacterial infections in diabetic foot ulcers and its antimicrobial susceptibility and resistance of the isolates were studied keeping these clinical difficulties in mind.

MATERIAL AND METHODS

This study was conducted on fifty diabetic male and female patients with foot ulcers, at tertiary care centre in Chennai, from October 2012 to February 2013. The maximum number of the subjects belonged to the age group 45 – 70 years, with the history of uncontrolled diabetes for long years. The patients were randomly selected; those who were on antimicrobials for at least past two weeks were excluded. The participants were included in the study after obtaining the informed consent from subjects and the ethical committee approval.

Ulcers were irrigated and washed with normal saline, superficial exudates debridement was done to avoid the overgrowth of contaminants. Three swabs collected from the inner side of the lesion were transported and processed for aerobic, anaerobic and direct smear respectively without delay. Nutrient agar, blood agar, MacConkey agar and Robertsons cooked meat media (RCM) were used for the bacterial culture and the inoculated culture plates were incubated at 37^0C for 24-48 hours. The identification of organisms was done by conventional biochemical tests and sugar fermentation test, colony morphology and Gram staining. Antimicrobial susceptibility test were performed according

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to Kirby- Bauer disc diffusion method as recommended by Clinical and Laboratory Standards Institute (CLSI- 2010, M100-S17).

RESULTS

Of the 50 patients studied, 56% were males and 44% were females. Out of the samples cultured, a total of 52 organisms were isolated (49 aerobic organisms and 3 anaerobes) which represent an average of 1.04 organisms per case. No bacterial pathogens were isolated in 3 samples. Aerobic bacteria accounted for 94% of the total isolates. Among these, the Gram negative bacilli obtained were 53.9% and the Gram positive cocci constituted 40.4%. *E. coli* was the common pathogen among the Gram negative bacilli followed by *Pseudomonas aeruginosa*, *Proteus* spp., and *Klebsiella* spp., comprising 19.2%, 15.4%, 9.6% and 9.6% respectively. Among the Gram positive cocci *S. aureus* was most frequently isolated organism, 21.2% of the total aerobic isolates. Anaerobic bacteria isolated were 6.0% of the total isolates, which includes *Bacteroides fragilis* and *Peptostreptococcus* spp. (Table-2).

Antibiotic susceptibility of *S. aureus* revealed that 36.4% of them were Methicillin resistant *S. aureus* (MRSA) and all were susceptible to vancomycin. All strains of *S. aureus* were sensitive to azithromycin. Most of the strains of *S. aureus* were susceptible to chloramphenicol, tetracycline, cefazolin and cefuroxime. However, most of the strains of *S. aureus* were resistant to penicillin and amoxicillin (graph-1). *E .coli, Klebsiella* spp. and *Pseudomonas* spp. were highly susceptible to amikacin (100%), but *Proteus* spp. were resistant to amikacin. For Gram negative bacilli antimicrobial susceptibility was variable with ceftriaxone, ceftazidime, ciprofloxacin and aztreonam. Further, all the anaerobes isolated were susceptible to metronidazole. Antibiotic susceptibility patterns of aerobic Gram negative and Gram positive bacteria are shown in the tables 3 and 4 respectively.

TABLE 1: Age distribution of the cases in the study

Age in years	No. of cases	Percentage
< 40	4	8%
41 – 50	11	22%
51 – 60	17	34%
61 – 70	13	26%
>70	5	10%

TABLE 2: Bacterial cultures isolated in the study

Isolates cultured	Number of isolates	Percentage (%)
S. aureus	11	21.15%
CoNS	4	7.7%
B-haemolytic streptococci	2	3.85%
Diphtheriods	3	5.76%
Enterococci	1	1.9%
E. coli	10	19.23%
P. aeruginosa	8	15.38%
Klebsiella spp.	5	9.62%
Proteus spp.	5	9.62%
Bacteroides fragilis	2	3.85%
Peptostreptococcus spp.	1	1.9%

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TABLE 3: Antibiotic susceptibility pattern of aerobic Gram positive bacteria

Antibiotic	*S. aureus* (n=11)	β-haemolytic Strep. (n = 2)	CoNS (n = 4)	Diphtheriods (n = 3)	Enterococci (n = 1)
P (10µg)	3 (27.3%)	-	1 (25%)	2 (66.6%)	1 (100%)
Am (10µg)	2 (18%)	-	1 (25%)	1 (33.3%)	1 (100%)
AmC	8 (72.7%)	2 (100%)	3 (75%)	2 (66.6%)	1 (100%)
Co(25µg)	3 (27.3%)	-	1 (25%)	1 (33.3%)	1 (100%)
Cp (30µg)	6 (54.5%)	2 (100%)	1 (25%)	2 (66.6%)	-
Cef (30µg)	10 (91%)	2 (100%)	3 (75%)	3 (100%)	1 (100%)
Cu (30µg)	9 (81.8%)	1 (50%)	3 (75%)	3 (100%)	1 (100%)
E (15µg)	8(66.6%)	2 (100%)	1 (25%)	1 (33.3%)	1 (100%)
Az (15µg)	11(66.6%)	2 (100%)	2 (50%)	1 (33.3%)	1 (100%)
Cf (5µg)	8 (72.7%)	-	2 (50%)	1 (33.3%)	1 (100%)
Of (5µg)	7 (63.6%)	2 (100%)	1 (25%)	1 (33.3%)	1 (100%)
C (30µg)	9 (81.8%)	2 (100%)	3 (75%)	3 (100%)	1 (100%)
T (30 µg)	10 (91%)	2 (100%)	2 (50%)	3 (100%)	1 (100%)
Pc (100µg)	9 (81.8%)	1 (50%)	2 (50%)	2 (66.6%)	1 (100%)

Note: P- penicillin, Am- amoxicillin, AmC- amoxicillin-clavulanic acid, Co- cotrimoxazole, Cp- cephalexin, Cef- Cefazolin, Cu- Cefuroxime, E-erythromycin, Az- azithromycin, Cf- ciprofloxacin, Of- ofloxacin, C- chloramphenicol, T- tetracycline, and Pc- piperacillin

GRAPH 1: Antibiotic susceptibility of oxacillin susceptible and oxacillin resistant *S. aureus*

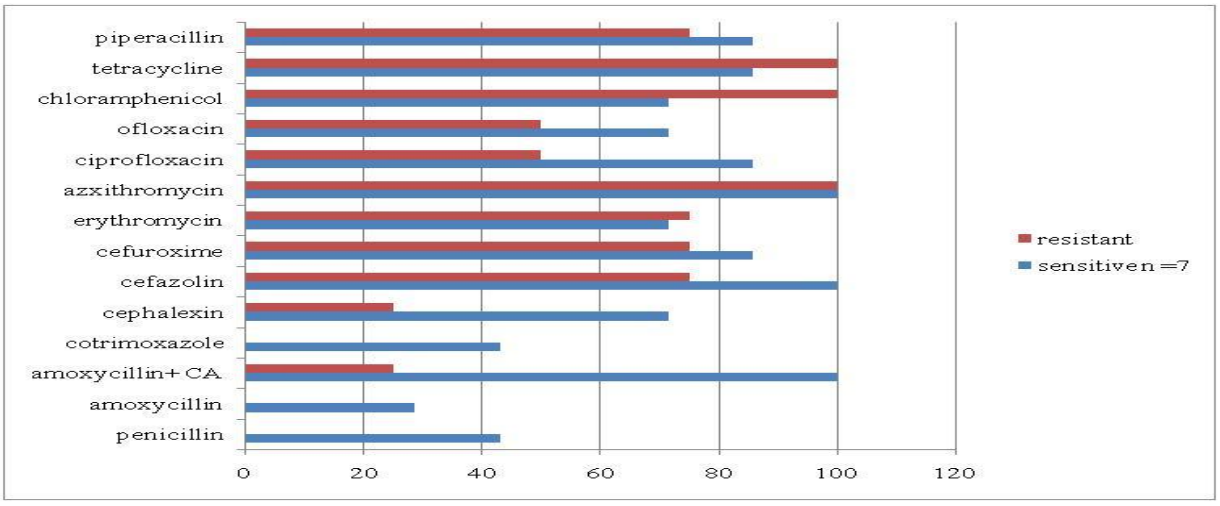

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TABLE 4: Antibiotic susceptibility pattern of aerobic Gram negative bacteria

Antibiotics	*E. coli* (n = 10)	*Pseudomonas* spp. (n = 8)	*Klebsiella* spp. n =5	*Proteus* spp. (n = 5)
Of (5µg)	2 (20%)	5 (62.5%)	3 (60%)	5 (100%)
Cf (5µg)	3 (30%)	7 (87.5%)	3 (60%)	4 (80%)
G (10µg)	3 (30%)	8 (100%)	3 (60%)	2 (40%)
Ak (30µg)	10 (100%)	8 (100%)	5 (100%)	1 (20%)
An (30µg)	5 (50%)	6 (75%)	4 (80%)	5 (100%)
Ce (30µg)	4 (40%)	4 (50%)	5 (100%)	1 (20%)
Ci (30µg)	4 (40%)	7 (87.5%)	5 (100%)	4 (80%)
Cu (30µg)	2 (20%)	3 (37.5%)	3 (60%)	3 (60%)
Ca (30µg)	6 (60%)	7 (87.5%)	4 (80%)	2 (40%)
Cx (5µg)	4 (40%)	2 (25%)	4 (80%)	3 (60%)
Cen (5µg)	2 (20%)	2 (25%)	3 (60%)	2 (40%)

Note: Of- ofloxacin, Cf- ciprofloxacin, G- Gentamicin, Ak- amikacin, An- aztreonam, Ce- cefotaxime, Ci- ceftriaxone, Cu- cefuroxime, Ca- ceftazidime, Cx- cefixime, and Cen- Cefdinir

DISCUSSION

DFIs are a major public health problem worldwide. The faster emergence of antimicrobial resistance to selective drug limits the usage of antibiotics to only clinically infected foot ulcers and to use the anticipated spectrum of antimicrobial[5]. On the other hand untreated DFIS may risk for limb loss. To overcome the unpleasant outcome of undermanaged diabetic foot[6] due to lack of knowledge of bacterial prevalence and therapeutic management this study was undertaken.

In the present study, 52 organisms were recovered from 50 patients with an average of 1.04 organisms per case. The findings were little lesser than that by Viswanathan *et al [*7]; where cultures yielded an average of 1.21 organisms per case. The rate of isolated pathogens per lesion was low compared to Lipsky *et al [*8] and Ramani *et a*l[9] who had reported the occurrence of mixed infections with an average of 2.1and 2.97 bacterial isolates per specimen respectively. The low prevalence of polymicrobial infection and low rate of isolated pathogens per lesion may be attributable to the lack of severity of most infections.

The study conducted by Slater *et al* showed the predominance of *S. aureus* in 50% of wound specimens [10]. On the contrary other studies reported that Gram negative bacteria were dominant in infected diabetic foot ulcers [3, 11]. The predominantly isolated Gram

negative bacteria (53.85%) in our study were *E. coli,* and *P. aeruginosa*. Almost similar results were obtained in Chincholikar *et al* [12] and in other Indian study [9]. *Klebsiella* and *Proteus* spp. were other important reported pathogens.

S. aureus, CoNS, β-hemolytic Streptococci, *Enterococcus* spp. and Diphtheriods were the Gram positive (40.38%) isolates. In our study *S. aureus* (21.15%) was predominantly isolated. The result was in accordance with the findings of previous study. Criado et al [13] and Chincholikar *et al*[12] also observed that *S. aureus* was the predominant bacterial pathogen accounting for 31.37% and 31.25% of the total aerobic isolates respectively, in their studies. Coagulase negative Staphylococci (CoNS) comprised 7.7% and displayed high degree of resistance to commonly used antibiotics, as previously reported[14,15]. CoNS were considered as wound contaminant. But now in various types of wound infections CoNS have been established as true pathogens, such as in noscomial prosthetic devices and catheter infectionsc [16]. In our study the MRSA were 7.7% of the isolates reported. Almost similar results have been reported in other study [17].

In this study the isolation rate of β-hemolytic Streptococci was low and it was only 3.85% of bacterial isolates, almost in concurrence with the study conducted by Chincholikar *et al*, where it was 5.63% [12].

Enterococcus spp. were considered low virulence commensals but opportunistic pathogens in compromised diabetic patients [17]. However, now *Enterococcus* spp. has been more often isolated in DFIs, In one study, it was reported that *Enterococcus* spp. were 29.0% of the isolates from the foot ulcers [18]. However in our study, it was reported only in 1.9%, which may not be a threat to the patients.

Diphtheriods were considered non-virulent spp., and still its pathogenicity in DFI is under debate. We found Diphtheriods in 5.76% of the isolates. Significance of diphtheriods in infected diabetic wound was focused by Bessman *et a*l [19]. Diphtheriods were also isolated from infected leg in other study [20].

Regarding the antimicrobial susceptibility pattern of Gram positive aerobic isolates, more than 85% were susceptible to cefazolin (90.5%), cefuroxime (85.7%), tetracycline (85.7%) and chloramphenicol (85.7%). *S. aureus* also showed good susceptibility to azithromycin (80%), piperacillin (71.5%) and amoxicillin-clavulanic acid (76.2%).

Among the Gram negative bacilli, more than 85% strains were susceptible to amikacin. Further, more than 65% strains of Gram negative bacilli were susceptible aztreonam (71.4%), ceftriaxone (71.4%) and ceftazidime (67.8%), but only 50.0% were susceptible to cefotaxime. The Gram negative bacilli also showed good susceptibility to ciprofloxacin (64.3%).

CONCLUSION

DFIs are a real public health problem worldwide. Diabetic patient with foot complications are highly vulnerable to amputation of foot or leg. *E. coli* and *P. aeruginosa*

were the most frequent Gram negative pathogens and *S. aureus* was the most common among Gram positive organisms. Combination of antimicrobial agents covering both gram positive and gram negative would be the right option for empiric treatment initially, rather than monotherapy. The choice of antimicrobials should be based on the severity of infection, the organisms isolated and the antibiogram.

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