Original Research Article

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Bacteriological study of diabetic foot ulcer according to Wagner's classification: a one-year study

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ABSTRACT

Background: Diabetes is considered as one of the most important and rapidly evolving non-communicable disease which requires top priority around the world. 10-25% of diabetics develop foot infections [DFI's] during the course of their disease period ranging from simple calluses to major Osteomyelitis. 3 Microbial infections of the diabetic foot ulcers are difficult to manage because of multiple factors associated in response including overall glycaemic control, associated complications like vascular disease and neuropathy. The present study was done to assess the microbial flora according to Wagner's classification for diabetic foot disease. To isolate, identify the aerobic bacterial pathogens and their drug sensitivity pattern with reference to ESBL production

Methods: A prospective study was conducted at a tertiary care hospital by department of general surgery among Diabetics presenting with diabetic foot infections. All the enrolled cases were classified based on Wagner's classification and socio demographic data was noted. Specimens from the ulcer were collected and processed for isolation, identification of pathogens based on standard CLSI guidelines. The antibiotic sensitivity of the isolates was done and ESBL production was demonstrated by standard guidelines.

Results: 346 cases were enrolled in study and grade-3 Wagner's ulcers were predominant. Males were predominant, 46-55 years age group was common with mean age of 55.3±1.4 years. Peripheral arterial disease was more common than peripheral neuropathy and both were found significantly associated with development of ulcers. Methicillin resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* were most common isolates. MRSA exhibited maximum sensitivity to vancomycin, clindamycin and linezolid. Gram negative isolates exhibited maximum sensitivity to meropenems, piperacillin-tazobactam. The prevalence of ESBL producers in the study was 16.24%.

Conclusions: DFI are a serious concern in uncontrolled diabetics and require proper management. Outcome of the infection depends upon the grading of the ulcer as per Wagner's classification and also the nature of the infection either polymicrobial or with a single organism. Our study highlights and suggests that prospective multicentre studies are required to assess the appropriate antibiotic regimen in diabetic foot ulcers and proper management of antibiotics must be implemented to decrease the incidence and development of multi drug resistant organisms.

Keywords: Diabetic foot infections, ESBL, Pseudomonas aeruginosa, Staphylococcus aureus, Wagner's classification

INTRODUCTION

Diabetes is considered as one of the most important and rapidly evolving non-communicable disease which requires top priority around the world. The prevalence of diabetes has increased tenfold globally from 1.2% to 12.1% between 1971 to 2000 and rising still.61.3 million people live with diabetes in India (2011 estimates) and expected to increase by 101.2 million by the end of 2030. The annual cost for India due to diabetes was about 38

billion U.S dollars in 2011.1,2 10-25% of diabetics develop foot infections [DFI's] during the course of their disease period ranging from simple calluses to major Osteomyelitis. Diabetic foot lesions are a major medical, social and economic problem and leading cause of hospitalization for diabetics. The major underlying cause for development of DFI's is peripheral neuropathy which affects 30% of diabetic population and leads to loss of protective sensation of pressure and pain, together with reduced joint mobility. The presence of macro vascular disease and associated infection of the ulcer increases the chances of amputation of lower limb.3 Microbial infections of the diabetic foot ulcers are difficult to manage because of multiple factors associated in response including overall glycaemic control, associated complications like vascular disease and neuropathy. The depth of the ulcer is an important factor which decides the outcome of the diabetic foot ulcer. Various wound classification systems are being used to assess the severity of diabetic foot ulcer and encompass different factors namely site, depth, presence of neuropathy, ischemia etc. However, an easy to use classification system, that provides uniform description of the ulcer and infection will guide in proper management plan strategy and to predict the outcome in term of healing and amputations.4 Wagner's system classification is the most widely used in grading of diabetic foot ulcers.⁵ Most of the authors assume that management of infections require identification of pathogenic microbial flora and initiate appropriate antibiotic therapy based on sensitivity report. Most of these infections are polymicrobial in nature and the pathogens depend upon the metabolic factors, foot hygiene and resistance pattern of organisms. Development of multidrug resistance is an important factor which hinders the improvement of DFI.6

The present study was done to assess the microbial flora according to Wagner's classification for diabetic foot disease. To isolate, identify the aerobic bacterial pathogens and their drug sensitivity pattern with reference to ESBL production.

METHODS

The present was conducted at a tertiary care hospital of south India by department of general surgery in association with Department of Microbiology for a period of one year from January 2015 to December 2015. The study protocol was approved by the institutional ethical committee and all the guidelines were followed. All the patients attending or referred to the diabetic foot clinic of general surgery department were enrolled in the study. Enrolled cases in the study were once again confirmed as diabetes by laboratory investigations. All the cases were admitted in the clinic and followed until discharge. The age, sex, duration of diabetes, duration of ulcer, type of ulcer, glycaemic control, associated complications like peripheral ischemia signs (intermittent claudication with or without peripheral pulses) were noted. Size of the ulcer was measured by multiplying the longest and widest diameters and expressed in centimeters squared. Assessment of the ulcer and grading as per the Wagner's classification was done by one General surgeon throughout the study to obviate inter observation bias. The Wagner's system assesses ulcer depth and grades as follows: grade-0 (pre-or post-ulcerative lesion), grade-1 (Partial/Full thickness ulcer), grade-2 (probing to tendon or capsule), grade-3 (deep with osteitis), grade-4 (partial foot gangrene) and grade-5 (whole foot gangrene) (Table 1).7 Ulcers were labelled infected if purulent discharge was present with signs of inflammation or lymphangitis / lymphadenopathy or edema. Osteomyelitis was diagnosed based on suggestive radiological findings or bone scan. All the cases were monitored until discharge.

Table 1: Wagner's classification of diabetic foot disease.

Grades	Symptoms
Grade-0	High risk foot and No ulceration
Grade-1	Superficial ulceration
Grade-2	Deep ulcer (Cellulitis)
Grade-3	Osteomyelitis with ulceration or abscess
Grade-4	Gangrenous patches
Grade-5	Gangrene of entire foot

Microbiological study

Specimens were collected from the ulcer sites after thorough washing the site with sterile normal saline and debridement. Sterile swab was used to collect the specimen from the base of the ulcer or deep portion of the wound edge with a sterile curette. Soft tissue specimens obtained were processed directly for aerobic bacteria. All the specimens were processed as per standard guidelines and isolation and identification of the isolates were done as per CLSI guidelines. Antibiotic susceptibility testing of the aerobic bacterial isolates was done by standard Kirby-Bauer disc diffusion method as per CLSI guidelines. Gram negative bacterial isolates were screened for ESBL production by using double disc diffusion method, methicillin resistant staphylococcus aureus by using oxacillin disc (1µg) and oxacillin screen agar (6µg/ml), Vancomycin resistant isolates were also detected by using vancomycin screen agar (6µg/ml) as per standard guidelines recommended by CLSI.8

Statistical analysis

All the data was entered into Microsoft excel spread sheet and checked for corrections. Quantitative variables were expressed as Mean±SD and quantitative variables as percentages.

RESULTS

A total of 386 patients attended the diabetic foot clinic during the study period and 346 required admission and fulfilled the inclusion criteria. 40 cases were on

antibiotics during the visit and were not included in the study. The baseline socio demographic characteristics, characteristics of the foot ulcers and grading of the ulcer were done during the period of admission. The socio demographic characters included the sex, age, duration of diabetes, type of diabetes, HbA1c, associated risk factors like smoking, alcoholism, hypertension, old history of DFI. Risk factors for development of foot ulcers like peripheral neuropathy, peripheral arterial disease, size of ulcer, site of ulcer was evaluated by clinical examination and noted.

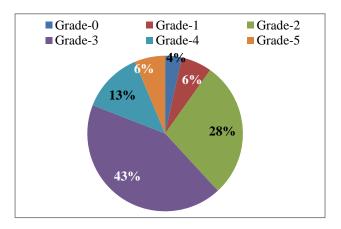


Figure 1: Distribution chart of cases based on Wagner's classification.

Figure 1 demonstrates the distribution of cases of DFI according to Wagner's classification, majority of the cases were in grade-3 (42.8%) followed in order by grade-2 (28.3%), grade-4 (12.7%) and equally distributed in grade-5 and 2 (6.4%).

Males were predominant in the study (71.7%) than females (28.3%). 42.8% of the cases were in the age group of 46-55 years followed by >56 years with 35.8% and 21.4% between 35 -45 years. The mean age of the males in the study was 53.6±1.8 years and females 56.4±1.6 years and mean age of the study subjects was 55.3±1.4 years. Majority of the subjects had type-II diabetes (95.4%). 43.4% of cases had a history of 6-10 years and only 16.2% with less than 5 years. The mean duration of diabetes among the cases in the study was 11.68±4.8 years. Smoking was observed in 61.3% and alcoholism in 58.4% of cases in the study (Table 2).

Table 3 summarizes the risk factors of the cases in the study. 61.3% of cases in the study had poor overall glycaemic control which is indicated by HbA1c levels >6.5%. Hypertension was observed in 86.1% of cases in the study and statistically significant association was observed. Majority of the cases had ulcer in the hind foot (48.6%), 35.8% in forefoot and 15.6% in mid foot. However, there was no significant association between site of the ulcer and development of DFI. 36 cases among 386 cases had no associated complications. Majority (37%) had peripheral arterial disease, 25.4% had peripheral nephropathy and 27.2% of cases had both. In

63.6% of cases the size of the ulcer was \geq 2.5 cm². Duration of the ulcer was <3 months in 65.3% of the cases.

Table 2: Socio-demographic data of cases enrolled in study.

Data Character	Number	Percentage
Sex		
Male	248	71.7
Female	98	28.3
Age group (years)		
35-45	74	21.4
46-55	148	42.8
>56 years	124	35.8
Type of diabetes		
Type-I	16	4.6
Type-II	330	95.4
Duration of diabetes		
≤ 5 years	56	16.2
6-10 years	150	43.4
>10 years	140	40.5
Smoking		
Yes	212	61.3
No	134	38.7
Alcoholism		
Yes	202	58.4
No	144	41.6

Table 3: Risk factors of cases in the study.

Risk factor	Number	Percentage
HbA1c	Titalinet	rereentage
<6.5%	144	41.6
>6.5%	212	61.3
Hypertension		
Yes	298	86.1
No	48	13.9
Site of ulcer		
Fore foot	124	35.8
Mid foot	54	15.6
Hind foot	168	48.6
Complications		
PN (peripheral neuropathy)	88	25.4
PAD (peripheral arterial disease)	128	37
Both	94	27.2
Size of ulcer		
≤2.5cm	126	36.4
≥2.5cm	220	63.6
Duration of ulcer		
≤3 months	226	65.3
>3 months	120	34.7

A total of 438 isolates were detected from 346 ulcer specimens. 82.7% (286/346) specimens produced only

pure culture of the organism, 8.1% (28/346) had infection with two organisms and 9.2% (32/346) had infection with three organisms, averaging 2.3 species per patient. Of the total 438 isolates detected from all the 346 cases of diabetic foot ulcers, majority (51.1%) were recovered from grade-3 ulcers, 23.7% from grade-2, 10% from grade-1, 7.3% from grade 4, 5% from grade 5 and 2.7% from grade-0 (Figure 2).

Infection with two or three isolates were observed mostly in grade-3 and 2 ulcers only.

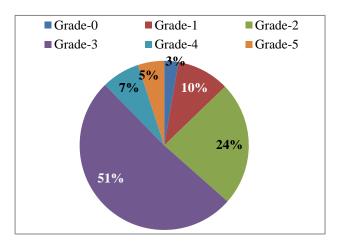


Figure 2: Distribution of isolates from DFU based on Wagner's grading.

Majority of the isolates were gram positive with 51.1% (224/438) and gram negative 48.9% (214/438). The profile of the organisms isolated is detailed in Table 4. Methicillin resistant *Staphylococcus aureus* [MRSA] was the predominant isolate in gram positive organisms with 19.2% frequency (84/438) followed in order by Coagulase negative staphylococcus (14.2%), Methicillin sensitive *Staphylococcus aureus*, *Enterococcus faecalis*, and Group B streptococci with 7.3%, 5.5% and 5% in the study.

Pseudomonas aeruginosa was the major isolate among Gram negative isolates with a frequency of 19.2% (84/438) followed by Klebsiella pneumoniae (11%), Escherichia coli, Acinetobacter baumanii (7.8%) and Citrobacter sp., Proteus sp. with 5% and 2.7% in the study. The results of antibiotic susceptibility of gram positive isolates are summarized in Table 5.

Maximum susceptibility of gram positive isolates was observed for vancomycin, linezolid, ticarcillin, clindamycin and mupirocin. All the isolates of group B *Streptococci* exhibited sensitivity to Penicillin. 100% sensitivity to vancomycin was exhibited by Enterococcus faecalis in our study.

Table 6 summarizes the antibiotic susceptibility pattern of gram-negative isolates in the study. Maximum susceptibility was observed with Meropenem for all the isolates in the study with cefoperazone-sulbactum,

Ticarcillin-clavulanic acid and Piperacillin-tazobactam the next. Extended spectrum β -lactamases [ESBL] production was studied for all the gram-negative isolates which were multi drug resistant.

Of all the 234gm negative isolates in the study, 76 were identified as multi drug resistant isolates and 38 of them identified as ESBL producers by double-disc diffusion method. The prevalence of ESBL producers in the study was 16.24%.

Pseudomonas aeruginosa and *Acinetobacter sp.* were the major ESBL producers in our study.

Table 4: Profile of bacteria isolated from diabetic foot infections.

Name of the isolate	Number	Percentage
Gram positive organisms		
Methicillin sensitive- Staphylococci aureus	32	7.3
Methicillin resistant Staphylococcus aureus	84	19.2
Enterococcus faecalis	24	5.5
Group B Streptococci	22	5.0
Coagulase Negative Staphylococci	62	14.2
Gram negative organisms		
Escherichia coli	34	7.8
Klebsiella pneumoniae	48	11.0
Pseudomonas aeruginosa	84	19.2
Acinetobacter baumanii	34	7.8
Proteus sp	12	2.7
Citrobacter sp	22	5.0

Table 5: Antibiotic susceptibility pattern of Gram positive isolates in the study.

Antibiotic	Proportion	ı susceptibl	e (No) (%)
	MSSA	MRSA	CONS
Penicillin	28 (87.5)	44 (52.4)	42 (67.7)
Amikacin	28 (87.5)	68 (81)	58 (93.5)
Ciprofloxacin	26(81.25)	59 (70.2)	48 (77.4)
Erythromycin	24 (75)	60 (71.4)	56 (90.3)
Tetracycline	20(62.5)	58 (69)	50 (80.6)
Clindamycin	32 (100)	80 (95.2)	62 (100)
Cotrimoxazole	20(62.5)	44 (52.4)	46 (74.2)
Netilmicin	30(93.75)	70 (83.3)	58 (93.5)
Linezolid	32 (100)	84 (100)	62 (100)
Ticarcillin	32 (100)	84 (100)	62 (100)
Mupirocin	30(93.75)	80 (95.2)	60 (96.8)
Amoxyclavulanic acid	28 (87.5)	56 (66.7)	54 (87.1)
Vancomycin	32 (100)	84 (100)	62 (100)

MSSA: Methicillin sensitive *Staphylococcus aureus*; MRSA: Methicillin resistant *Staphylococcus aureus*; CONS; Coagulase Negative *Staphylococcus*

DISCUSSION

Diabetic foot infections are one of the most threatening and difficult complication which a diabetic individual encounter during his uncontrolled glycaemic control with associated other complications. Different classification systems like Wagner's and University of Texas classification systems are available to classify DFI. In the present study Wagner's classification has been used to grade the ulcers of DFI and to assess the bacteriological profile of the ulcer according to the grade.

Table 6: Antibiotic susceptibility pattern of Gram Negative isolates in the study.

Table-6: Antibiotic susceptibility pattern of Gram Negative isolates in the study						
Antibiotic	Proportion Susceptible (No) (%)					
	E. coli	K. pneumoniae	P. aeruginosa	A. baumanni	Proteus sp.	Citrobacter sp.
Ciprofloxacin	24 (70.6)	30 (62.5)	44 (52.4)	24 (70.6)	6 (50)	14 (63.6)
Cefotaxime	26 (76.5)	34 (70.8)	54 (64.3)	26 (76.5)	8 (66.7)	18 (81.6)
Ceftazidime	26 (76.5)	34 (70.8)	56 (66.7)	22 (64.7)	6 (50)	14 (63.6)
Cefixime	28 (82.4)	36 (75)	62 (73.4)	24 (70.6)	6 (50)	16 (72.7)
Amikacin	28 (82.4)	32 (66.7)	68 (81)	28 (82.4)	7 (58.3)	16 (72.7)
Meropenem	34 (100)	44 (91.7)	80 (95.2)	30 (88.2)	10 (83.3)	22 (100)
Piperacillin	30 (88.2)	35 (72.9)	74 (88.1)	28 (82.4)	8 (66.7)	19 (86.4)
Piperacillin- tazobactam	30 (88.2)	36 (75)	74 (88.1)	28 (82.4)	10 (83.3)	19 (86.4)
Ticarcillin-clavulanicacid	32 (94.1)	36 (75)	78 (92.9)	30 (88.2)	10 (83.3)	19 (86.4)
Cefoperazone-sulbactam	30 (88.2)	42 (87.5)	72 (85.7)	32 (94.1)	10 (83.3)	21 (95.5)

In the present study, males were predominant than females as observed in many studies globally. Male population is more exposed to harder work than females with higher risk of trauma in their work zone. In our study males were 71.7% which is similar to the findings of Jeffcoate EJ who also reported a higher incidence of DFI in males with 67% in his study report. 9 Majority of the cases in the study were placed in Grade-3 in our study which is also on par with the findings of Armstrong DJ and Shea JD who reported that 48% of ulcers as grade-3 in their studies. 10,11 However findings of our study were in contrary to reports of Mayfeild JA who reported that 52% of ulcers were of grade-2 in his study. 12 Type-II diabetic patients were common in our study as observed in many studies and the incidence was around 95.4% which is almost similar to many studies in India. DFI were more common in cases with duration of diabetes >10 years in our study which was also reported by Yonem A in his study that more the duration more the development of DFI.13 Poor glycaemic controls with raised levels of HbA1c, long duration of hypertension were significant risk factors for development of DFI in our study and statistically significant association was found with these risk factors. Similar findings were reported by Lavery LA and Oyibo SO in their studies. 14,15 Incidence of Peripheral neuropathy in our study was 25.4% as similar to findings of Gershater MA who reported the incidence as 27% in his study and peripheral arterial disease as 40% which is similar to 37% in our study.16 Statistically significant association was found with PN and peripheral arterial disease in our study as similar to many others. Other factors like smoking, alcoholism, site of the ulcer, past history of treatment for DFI had no significant association with development of

DFI in our study. Few studies reported that smoking, and ulcer on the pressure sites like fore foot and hind foot are more common sites for development and were found statistically significant. Most of the studies state that size and depth of ulcer are important factors in the outcome of the DFI. Osteomyelitis of the bone is considered an important factor associated with high risk of amputation and poor outcome in DFI.

Gram positive isolates were more common than gram negative isolates in our study which is similar to the findings of Baba M in his study but contrary to the findings of Viswanathan V who reported gram-negative pathogens as most common pathogens in DFU.^{17,18} The differences in the study region, age group, study settings might be the reason for differences in the prevalence of pathogens. In our study Staphylococcus aureus which was methicillin resistant was the most common grampositive isolate followed by Pseudomonas aeruginosa among gram negative. These findings were similar to many studies in southern India but contrary to some of the studies in northern parts who reported Escherichia coli as the most common gram-negative pathogen and Coagulase Negative Staphylococci as gram positive pathogen. Polymicrobial infections were common among Grade-3 Wagner's DFU than others. This was similar to the findings of Shankar E et al who reported much higher incidence (32%) of polymicrobial infections in his study and Klebsiella pneumoniae was the most common pathogen in his study which was contrary to the findings of our study.19

In present study there was an equal distribution of group B *Streptococci* and *Enterococcus sp.* with 5% incidence,

few studies reported higher prevalence of Enterococci than group B Streptococci. This can be explained by the reason that differences in the site of the ulcer and prevalence of the pathogen in the region influences the type of the isolate from the ulcer. Coagulase Negative Staphylococci was one among the isolates in the study with a prevalence of 14.2%, this shows an increasing trend in the emergence of CONS as an important pathogen in DFI alone or as a part of polymicrobial infections. Among the gram-negative isolates, Klebsiella pneumoniae was next to Pseudomonas aeruginosa and was resistant to most of the commonly used drugs. Acinetobacter baumanii has gained importance as a multidrug resistant pathogen and is serious concern among DFIs. Findings of our study were mostly coinciding with the reports of many studies universally stating Klebsiella, Acinetobacter and Proteus sp. as most important pathogens implicated in serious infections of the diabetic foot ulcer.²⁰ The antimicrobial susceptibilities of the gram-positive isolates exhibited fully susceptibility pattern to vancomycin, linezolid and clindamycin with next most active drugs as netilmicin, amikacin, amoxyclavulanic acid. MRSA has been a pathogen of concern in DFI for past two decades. All the strains were susceptible to clindamycin, vancomycin and linezolid.

Multidrug resistance is a serious concern among gram negative isolates due to the production of extended spectrum B-lactamases. In our study *Pseudomonas aeruginosa* was the major ESBL producer with 15% followed by *Klebsiella pneumoniae* and *Acinetobacter baumanii*. Most of the studies universally are majorly concerned with the development of drug resistance due to ESBL production which is also observed in our study. MDR isolates were susceptible to meropenem, piperacillin-tazobactam and ceoperazone-sulbactum in our study. Few studies reported the emergence of resistance of these strains to carbapenems also.²¹

CONCLUSION

To conclude, DFI are a serious concern in uncontrolled diabetics and require proper management. Outcome of the infection depends upon the grading of the ulcer as per Wagner's classification and also the nature of the infection either polymicrobial or with a single organism. The present study highlighted the bacteriological profile of the diabetic foot infections with regard to Wagner's grading of ulcer and associated risk factors in management and outcome of the ulcer. Present study highlights and suggests that prospective multicentre studies are required to assess the appropriate antibiotic regimen in diabetic foot ulcers and proper management of antibiotics must be implemented to decrease the incidence and development of multi drug resistant organisms.

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