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Original Research Article

Bacteriological Profile and Their Antimicrobial Susceptibility from Diabetic Foot Infections in A Tertiary Care Centre From Kancheepuram, India

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Abstract

Diabetic foot infection is one of the most common complications of Diabetes and it is a major public health problem that leads to amputation if not treated. Moreover screening of the ulcers for microbial growth and antibiotic susceptibility will enable to initiate the appropriate antibiotic therapy. The present study was carried out to identify the profile bacterial pathogens from the diabetic foot ulcers and also to determine it's antibiotic susceptibility pattern. Ninety four patients with diabetic foot ulcer attending Surgery outpatient department were included; demographic and clinical examinations was done by the surgeons and the ulcers were assessed as per the Wagner classification of ulcers. Wound swabs and pus were collected from the diabetic foot infections and were processed using standard Microbiological techniques. The results revealed *Pseudomonas aeruginosa* predominated followed by *Klebsiella pneumoniae* and *Escherichia coli* among negative groups whereas *Staphylococcus aureus* dominated [two strains were Methicillin Resistant *Staphylococcus aureus* (MRSA)], followed by *Enterococcus* spp and *Streptococcus* among Gram positive. The Gram positive bacteria showed resistance to cephalosporins, aminoglycosides and other first line drugs. ESBL production was observed maximum in *Klebsiella* spp followed by *Proteus* spp and *Escherichia coli*. Metallo β lactamases production for *Pseudomonas* spp was found to be positive among 12 isolates. Hence by performing culture and sensitivity of diabetic foot ulcers will enable the antibiotic sensitivity pattern which will be helpful in determining the drugs for the empirical treatment thereby preventing indiscriminate use of broad spectrum antibiotics.

Keywords: Diabetic foot, bacterial profile, antimicrobial susceptibility, Wagner grade.

INTRODUCTION

The raising prevalence of diabetes leads to increase in its complications thereby around 415 million people were known to have diabetes [1]. Diabetes along with its complications is expected to result in increasing morbidity, mortality and health expenditure due to the requirement of specialized care [2]. Diabetic foot ulcer (DFU) is considered as a major and frequent complication of diabetes mellitus (DM) that is caused due to diabetic neuropathy and significantly increases the treatment expenses [3].

Globally, the developed countries have the DM with approximately 8.3% of the population and more than 79 million people have prediabetes, whereas the complication load in developing countries like India are very high approximately 42% [2, 4]. Lack of proper health infrastructure delays the time the patient to be seen and increases the risk of foot amputation. If the patient is poor, then he may not be able to afford the cost of repeated physician visits. In addition to these factors, smoking, tobacco use in any form and alcohol use increase the risk of diabetic foot ulcer [1, 5, 6].

In India, nearly 40 million people are diabetics and their socioeconomic status is poor. Diabetic foot infections are seen in 20% of the patients and hence are the most commonly faced clinical issues to treat. It was well analyzed that the diabetes persons are having high risk (12–25%) of developing a foot ulcer during their lifetime [7, 8]. The opportunistic infections are found as the most common cause of morbidity and mortality and are recorded between 40 and 80% of the diagnosed cases [9, 10]

Diabetic foot is one of the most significant and devastating complication of diabetes and is defined as a group of syndromes in which neuropathy, ischemia and infection lead to tissue breakdown, and possible amputation [7, 11]. Around 15% of diabetic patients will develop foot ulcers in their life time and this is known to precede amputation in 85% of the cases [1,

4]. The diabetic foot ulcers are difficult to heal as the wound does not get enough nutrients or oxygen from blood, leading to the risk of lower limb amputation.

Predominantly, soft tissue and bone infections of the foot are largely identified among diabetic patients who are hospitalized than the non-hospitalized individuals [12]. Both aerobic and anaerobic pathogens form the etiology for diabetic foot infections [13]. Gram positive bacterial pathogens play a prime role in diabetic foot ulcers followed by gram negative bacilli and few anaerobic pathogens that are mainly associated with ischemic or necrotic wounds [14]. In most of the studies, the frequency of the bacterial pathogens like *Staphylococcus aureus, Enterococcus faecalis, Pseudomonas aeruginosa, Escherichia coli, Proteus mirabilis, Acinetobacter baumanni, Klebsiella pneumoniae* etc [15].

Foot ulceration in diabetes is known to represent a major cause of morbidity and mortality, and carry considerable financial implications for healthcare organisations [16]. Diabetic foot ulcers and infections are closely associated with poor clinical outcomes for the patient and very expensive for both the patients and health care system. These infections lead to severe lower extremity amputations followed by disability of the patients, significant morbidity, increased stay of hospitalization and even death with multi organ dysfunction to multi organ failure [1, 4, 14].

Currently, increased cases with multidrug resistant (MDR) bacterial pathogens are commonly reported and long term infections have become the major cause for the amputations [8, 10, 12]. Cross transmission of such infectious moiety in community and hospital environment are aggressively presented thereby hygienic measures and isolation precautions may support the earliest healing of the wounds and ulcers [14, 17]. The three dimensional approach for the management of diabetic wound infections are microbial species, biofilming ability and respond to antibiotics. Therefore, this cohort study was designed to identify and determine the bacterial pathogens from the diabetic foot ulcers and the antibiotic susceptibility pattern.

MATERIALS AND METHODS

Study Design

This prospective study was conducted in the Department of Microbiology, Saveetha Medical College and Hospital, Kancheepuram District of Tamilnadu from October 2017 to June 2018. Ninety four patients with diabetic foot ulcer attending surgery outpatient department were included in this study. Study was initiated after getting Institutional ethical clearance and informed consent was obtained from the subjects in the vernacular language.

Sample Collection and Processing

Detailed history was taken from the patients after thorough clinical examination was done by the Surgeons and they also assessed the ulcers as per the Wagner classification of ulcers. After the area of the wound had been cleaned using 0.9% sterile saline and debrided, swabs were collected from the ulcer by rotating the swab over a $1cm^2$ of the wound for 5 seconds using sufficient pressure to extract fluid from the inner part of the wound [18]. Care had been taken that no antimicrobial agent or antiseptic was introduced into the wound before specimen collection. Two swabs were collected from the depth of the ulcers. Out of the two swabs collected, one was used for microscopic examination like Gram stain and other for culture.

The specimens were placed into sterile transport containers and sent to the microbiology laboratory for Gram's staining and aerobic culturing and sensitivity within 30 minutes. Anaerobic culturing was not performed in this study. Gram-staining was performed from the sample and the smear examined. Swabs were further inoculated on Blood agar, Chocolate agar, MacConkey agar and Thiogycollate medium and plates were incubated at 37°C for 24 hours. The colonies were determined for its specific determination including colony morphology, staining reactions and biochemical reactions by using standard techniques [19]. Antibiotic sensitivity was done using Kirby Bauer's disc diffusion technique method as described in the Clinical Laboratory Standard Institute (CLSI) guidelines [20, 21]

RESULTS

The demographic profile in this study showed that out of 94 patients enrolled for this study, 69 patients were males and 25 patients were females and the age ranged from 31 to 76 years. The maximum cases were observed in the age groups between 41 and 60 due to their high frequency of diabetes and its complications and also exposure to various related co-morbid diseases, disorders and complications. Demographic and clinical data of patient with diabetic foot ulcer which includes mean duration of diabetes, age, sex, duration of hospital stay, amputation and associated comorbid conditions were included in (Table-1).

Table-1: Demographic and clinical data of diabetic foot patients

Parameter	Values	Range (%)
Mean duration of diabetes		10 years
Age	< 40 40-60 >60	10 (10.6%) 64 (68%) 20 (31.2%)
Sex	Male Female	69 (73.4%) 25 (26.5%)
Duration Of Hospital Stay	1-6 days 1 week 2 weeks 3 weeks 1 month >1 month	5 (5.4%) 24 (25.5%) 42 (44.7%) 8 (8.5%) 13 (13.8%) 2 (2.1%)
Wagner grading of ulcer	0 1 2 3 4 5	0 51 (54.2%) 31 (32.9%) 3 (3.1%) 7 (7.4%) 2 (2.1%)
Amputation		3 (3.1%)
Associated comorbid conditions	Hypertension Neuropathy Nephropathy	32 (34%) 12 (12.7%) 8 (8.5%)

Further the wounds were analyzed for its types thereby it was found that all the patients had foot ulcers (100%) followed by gangrene (5.3%), observation of maggots (3.2%), maggots with foul smell (1.1%), abscess (1.1%) and non healing wound (3.2%) (Figure-1). Other data like the number of days of hospitalization, types of surgery, history of diabetes and wound grading were collected.

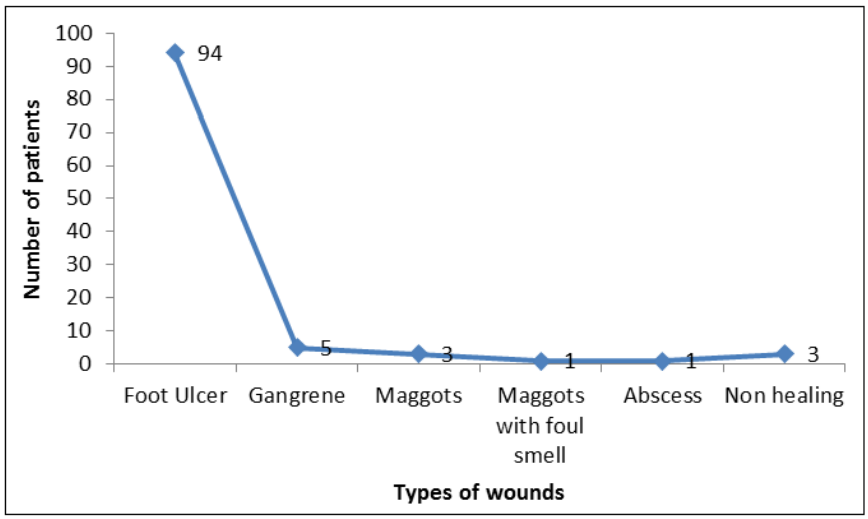

Fig-1: Types of wounds recorded

It is the known factor that the length of hospitalization may increase various physical, physiological and psychological disturbances to the patients due to non-independent activities. This study also analyzed the duration of the hospital stay which is the prime factor for the non-healing or poor prognosis of diabetic foot ulcers. Around 70% of people were staying around two weeks and above where as the rest of the people had stayed less than two weeks.

The evaluation and classification of diabetic foot ulcers are essential in order to organize the appropriate treatment plan and follow up. The wounds were classified according to the Wagner-Meggitt classification consisting of 5 wound grades and the details were represented in which depicts the number of subjects having the grades of ulcers thereby grade 1 dominated with 54.3% followed by grade 2 (33%).

The diabetic foot ulcers need extensive debridement down to viable tissue and should be left

open until healthy granulation tissue has formed; repeated debridement may be necessary. In our study wound debridement was done in 78 patients, disarticulation in 9 patients and amputation in 3 patients which is shown in (Table-2).

Table-2: Types of surgery

Types of surgery	Number of cases (n=94)	Percentage
Dysarticulation	9	9.6
Wound debridement	78	83.0
Amputation	3	3.2
Fasciotomy	1	1.1
Split skin grafting	1	1.1
No surgery	1	1.1

As far as the microorganisms are concerned, among 94 bacterial isolates, 69 isolates (73.4%) were gram-negative and 25 (26.6%) were gram-positive. *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* were the most commonly isolated bacterial pathogens with 22.3% and 17.0% respectively followed by 19.1% of *Staphylococcus aureus* which is shown in (Table-3).

Table-3: Bacterial isolates of pus samples from diabetic foot ulcers

Bacterial isolates	No. of isolates	Percentage
Gram positive bacteria		
Staphylococcus aureus	18	19.1
Enterococcus spp.	06	6.4
Streptococcus spp.	01	1.1
Gram negative bacteria		
Klebsiella pneumoniae	16	17.0
Escherichia coli	13	13.8
Pseudomonas aeruginosa	21	22.3
Proteus mirabilis	09	9.6
Acinetobacter spp.	05	5.3
Klebsiella spp.	02	2.1
K. oxytoca	01	1.1
Proteus hausseri	01	1.1
Morganella morganii	01	1.1

The antibiotic susceptibility tests were performed and the sensitivity patterns of all the 13 different bacterial isolates were analyzed. As shown in (Table-4), all the Gram-positive bacteria showed moderate sensitivity to most of the antibiotics. *Staphylococcus aureus* showed good sensitivity for erythromycin, linezolid and vancomycin. Among 18 isolates of *Staphylococcus aureus*, two strains were identified as Methicillin Resistant *Staph aureus* (11%). No resistance found among the *Streptococcus* isolates. Most of the *Enterococcus* sp showed sensitivity for Vancomycin, Linezolid, Ampicillin, High level Gentamycin and Co-trimaxazole.

Table-4: Antibiotic resistant pattern of gram positive organisms (25) isolated from diabetic foot patients

Antibiotic	Total resistant strains
Ampicillin	6 (24)
Ciprofloxacin	9 (36)
Cefoxitin	2 (8)
Cotrimaxazole	6 (24)
Erythromycin	7 (28)
Gentamycin	11 (44)
Linezolid	0
Ofloxacin	8 (32)
Tetracyclin	11 (44)
High level resistant to gentamycin	4 (16)
Penicillin	19 (76)
Vancomycin	0

[Figure in parenthesis denoted percentages]

Overall the Gram negative bacteria showed good activity against cephalexin, colistin, meropenem, polymyxin B as shown in Table-5. The maximum resistance was observed in *Klebsiella pneumoniae, E. coli, Pseudomonas aeruginosa, Proteus mirabilis, Acinetobacter baumanii* and *Morganella morganii* showed maximum resistance to ceftazidime, cefazolin, cotrimaxazole, cephelothin etc (Table-5).

Table-5: Antibiotic resistant pattern of gram negative organisms (69) isolated from diabetic foot patients

Antibiotics	Total resistant strain
Ampicillin	52 (76.4)
Amikacin	35 (36.4)
Ceftazidime	43 (63.2)
Cefixime	11 (16.1)
Cefaperazone-Sulbactam	56 (82.3)
Ciprofloxacin	32 (47)
Cotrimaxazole	53 (77.9)
Colistin	6 (8.8)
Cefepime	64 (94.1)
Doripenem	42 (61.7)
Gentamycin	44 (64.7)
Imipenem	41 (60.2)
Meropenem	2 (2.9)
Ofloxacin	38 (55.7)
Polymyxin B	10 (14.7)
Piperacillin-Tazobactam	33 (48.5)

[Figure in parenthesis denoted percentages]

ESBL production was observed in 9 isolates of *Klebsiella* spp (47.3%), 4 isolates of *Proteus* spp (40%), 7 isolates of *Escherichia coli* (53.8%). MBL production for *Pseudomonas* spp was found to be positive for 12 (54.8%) which is shown in (Figure-2).

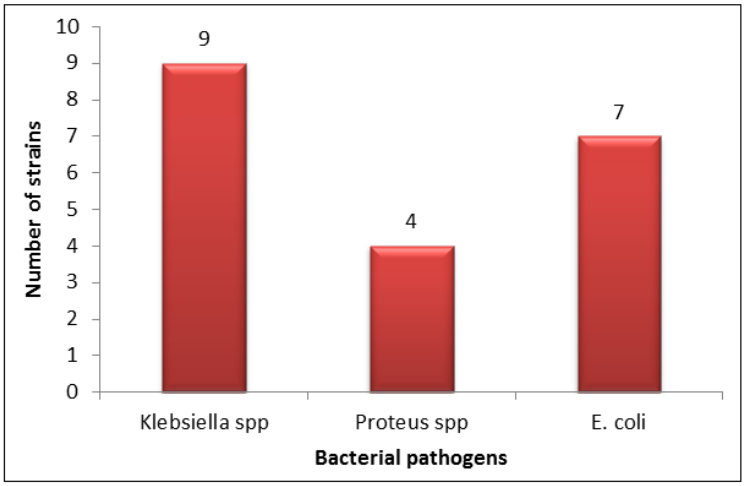

Fig-2: Percentage of ESBL Positive in *E. coli, Klebsiella spp, Proteus spp*

DISCUSSION

The annual incidence (population-based) of diabetic foot ulcers is estimated to be 1.0–4.1%, while the lifetime rate extends to around 25%. Foot related complications among patient with diabetes have become an increasingly significant public health concern in both the developed and developing countries. In order to avoid or reduce the detrimental consequences associated with diabetic foot ulcers, patient history related treatment approaches and role, and opinion of family physician must be implemented. Many of the etiological factors contributing to the formation of diabetic foot ulceration may be identified using simple, inexpensive equipment in a clinical setting [13, 20, 22-24].

This study presents the clinical, microbiological and co-morbid conditions related to patients with diabetic foot ulcers. Among the 94 patients included in this study, 69 were males and 29 were females, majority of patients (55%) were in the age group of 41 to 60 years. It is also proved that more number of patients with diabetes mellitus between the same age group [25, 26]. Duration of diabetes mellitus

is also contributing factor for development of diabetic foot ulcer as seen in our study.

The duration of the hospital stay was analyzed in this study thereby maximum cases with 42 were found among 2 weeks hospital stay followed by one week and one month with 24 and 13 cases respectively which was proved in other studies as well [6, 12, 27]. The data from North India suggest that one-third of patients with diabetes have prevalent peripheral neuropathy related to long duration hospital stay [28]. More importantly, two-thirds of the patients were at risk for foot ulcers and 9% had prevalent ulcer, out of which 20.2% required amputation due to nosocomial and iatragenic infections. In India, the geriatric cases maintained at home is much critical, thus the attenders admitted the cases and allowed them to stay longer, which is much lower than reported in the Western world [25, 26].

Majority of patients in the present study presented to the surgical department between few weeks and 24 weeks (median of 12 weeks) of onset of an ulcer. Late presentation in our patients may be attributed to low socioeconomic status, poverty, lack of diabetes education (regarding the importance of general foot care, the significance of diabetes and its complications), unrecognized foot trauma from walking barefoot and lack of access to medical care. Other contributing factors for late presentation include attempts at home surgery, trust in faith healers and undetected diabetes [7, 14, 23].

Grading of the ulcers is also an important task to be analyzed in the diabetic foot ulcer cases thereby this study predominantly registered the maximum cases of 51 in grade 1 followed by 31 in grade 2, 7 cases in grade 4 and 2 cases in grade 5 which was well correlating with the study conducted by [29]. The grading of the DFU cases are low prevalence in Indian studies, due to under-reporting, younger age and shorter duration of diabetes.

One of the most common complications of these ulcers is infection, if left untreated, results in the need for distal limb amputation [24]. In our study, 3 patients were amputated in which there duration of having diabetes was an average of above 10 years and all the 3 patients had grading of ulcer 5 which is classified according to wagner classification.

Diabetics with ulcers commonly experience infection with Gram negative organisms like *Pseudomonas aeruginosa*, *Escherichia coli*, *Klebsiella species*, *Proteus species* etc. and anaerobes and these organisms also show multi-drug resistance which was proved in the study [24]. In this study, among Gram negative bacterial pathogens, *Pseudomonas aeruginosa* dominated with 21 isolates, *Klebsiella pneumonia* dominated with 16 isolates followed by *Escherichia coli*, *Proteus mirabilis* and *Acinetobacter baumanii* with 13, 9 and 5 samples respectively

Among the gram positive bacterial isolates, *Staphylococcus aureus* contributed among 18 patients and similar finding was seen in other study [4, 14, 30]. Other studies highlighted that the Streptococci were cultured from more number of patients, with *S. agalactiae* comprising almost half of the strains. *In this study, Enterococcus species were isolated from 6 samples thereby it was found second top among Gram positive bacterial isolates.* Enterococcal isolates showed higher resistance for erythromycin, tetracycline, gentamycin, penicillin and ofloxacin and this was correlated with some other study [5, 27, 31].

Previous use of antimicrobial drug may increase the prevalence of *Enterococcus* spp. in diabetic foot infections. The increased prevalence of *Enterococci* has now emerged as a public health concern. In general, both Gram positive cocci and Gram negative bacilli cause diabetic foot infections and this study showed a preponderance of Gram negative bacilli. There was a variation in the bacterial species of the diabetic foot infections based on the geographical location [4, 21, 31].

To conclude, knowledge on the antibiotic susceptibility pattern of the isolates from diabetic foot infections is crucial for planning the appropriate treatment of these cases, prior to getting the susceptibility reports from the laboratory. Thus this study highlighted the importance of grading of ulcers, laboratory report for choosing the appropriate antibiotics and its concentrations for the effective therapy. In most cases, the patients are dehospitalized before the laboratory reports are received. From this study, it is clearly evident that the knowledge on the antibiotic sensitivity pattern of the isolates will be helpful in determining the drugs for the empirical treatment of diabetic ulcers. Moreover, there is an urgent need for continuous surveillance of resistant bacteria to provide the basis for empirical therapy and reduce the risk of complications.

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