

Diabetic foot ulcers at a tertiary care hospital: A clinico-microbiological profile

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Abstract

Introduction: Diabetic foot ulcer is one of the most common complications and cause of morbidity in patients with diabetes mellitus (DM). This study aims to determine the clinical and microbiological profile of diabetic foot ulcers and to determine their susceptibility pattern.

Materials and Method: The present study was conducted in the Department of Microbiology at a tertiary care centre. 150 patients with diabetic foot ulcers were included in the study over a period of two years between December 2014 to December 2016. Appropriate samples were collected and processed. Isolation and identification of bacterial and fungal cultures was done by conventional methods and antimicrobial susceptibility pattern was determined. Correlation between diabetic history, ulcer duration, grade of ulcer (assessed by Wagner scale) and rate of bacterial and fungal isolation was made. Statistical analysis was done by Chi square test, Fischer Exact test and Single proportion Z test using SPSS software.

Results: In our study, 138/150 samples were culture positive (47 polymicrobial and 91 monomicrobial). A total of 207 isolates out of 150 samples were isolated (175 bacterial and 32 fungal isolates). *Pseudomonas aeruginosa* 27.4 % (48/175) followed by *Staphylococcus aureus* 19.4 % (34/175) were most common bacterial pathogens isolated. 21.3 % (32/150) of the samples were positive for fungal growth, of which *Candida albicans* 25 % (08/32) was most common fungi isolated. Significant fungal isolation (*p* value 0.023) was made from samples showing no bacterial growth and fungal positivity was significantly (*p* value 0.39) associated with long standing non healing grade IV ulcers.

Conclusion: Our study stresses on the need to study the clinico- microbiological profile of diabetic foot ulcers and to determine their susceptibility pattern as prompt treatment will prevent devastating consequences such as sepsis and amputation.

Keywords: Diabetes mellitus, polymicrobial infection, diabetic foot ulcer.

Introduction

Infected diabetic foot ulcers are a challenge to the treating clinician as it takes dedication and strict vigilant treatment, however it most often leads to non traumatic lower extremity amputation.⁽¹⁾ The diabetic foot ulcer serves as a niche for polymicrobial infection and multidrug resistant organisms and is often associated with inadequate glycemic control.⁽²⁾ This study was taken up to emphasize the need to determine the microbiological profile and antimicrobial susceptibility pattern of organisms isolated from patients with diabetic foot ulcers. The risk factors and outcome of these infections were also studied.

Materials and Method

150 diabetic patients attending surgery Out patient department (OPD) and admitted to the General surgery ward for diabetic foot ulcer management at a tertiary care hospital were included in the study over a period of two years between December 2014 to December 2016. Age, sex, type and duration of diabetes, treatment, compliance and personal habits were recorded. Meticulous clinical assessment of the ulcer (site, size, shape, grade, classification) and signs of infection (swelling, exudate, surrounding cellulitis, odour, tissue necrosis, crepitation and pyrexia) was made. Grade of ulcer was assessed using the Wagner classification (Table 1).^(3,4)

The ulcer was cleaned with povidone iodine solution and sterile normal saline followed by debridement of superficial exudates. Tissue samples were obtained from depth of the ulcers and edge of the ulcer, consisting mostly the granulation tissue and necrotic slough found on the ulcer bed. Average size of the tissue block collected measured around 0.5×0.5cms. Tissue samples thus collected were transferred aseptically into autoclaved plastic bottles containing 4-5ml of sterile normal saline which was then labelled and promptly brought to the Microbiology lab. At the lab, the tissue samples were churned using a sterile triturer and was subjected to the following processes:- 10% KOH Mount, Gram stain, inoculation onto 5% Sheep Blood agar, Mac conkey agar and Sabouraud's dextrose agar with antibiotics and with and without cycloheximide. Identification of bacterial isolates by conventional biochemical tests, examination for Germ tube formation and streaking on Chrome agar (HIMEDIA, India) from growth showing Gram positive budding yeast like cells and Lactophenol cotton blue mount (LPCB) for mycelial forms.

Antibiotic susceptibility pattern of the bacterial isolates was determined by Kirby-Bauer disk diffusion method according to CLSI 2014 guidelines.

MRSA detection: Detection of MRSA was done by using a cefoxitin (30 µg) disc. Those isolates which produced a zone of inhibition which was less than or equal to 21 mm were considered as Methicillin

Resistant *Staphylococcus aureus* (MRSA). According to CLSI 2014 guidelines.

Metallobetalactamase (MBL) detection: MBL detection was done using Imipenem (10 µg) and Imipenem EDTA (10 µg / 750 µg) combined disc diffusion test. A difference in the zone of inhibition of > 7mm around the Imipenem - EDTA disc as compared to the zone around Imipenem disc were considered as MBL producers.⁽⁵⁾

Follow up was done upto six months or till ulcer healing in collaboration with the Department of Surgery.

Results

Our study involved 150 diabetic patients with infected foot ulcers.

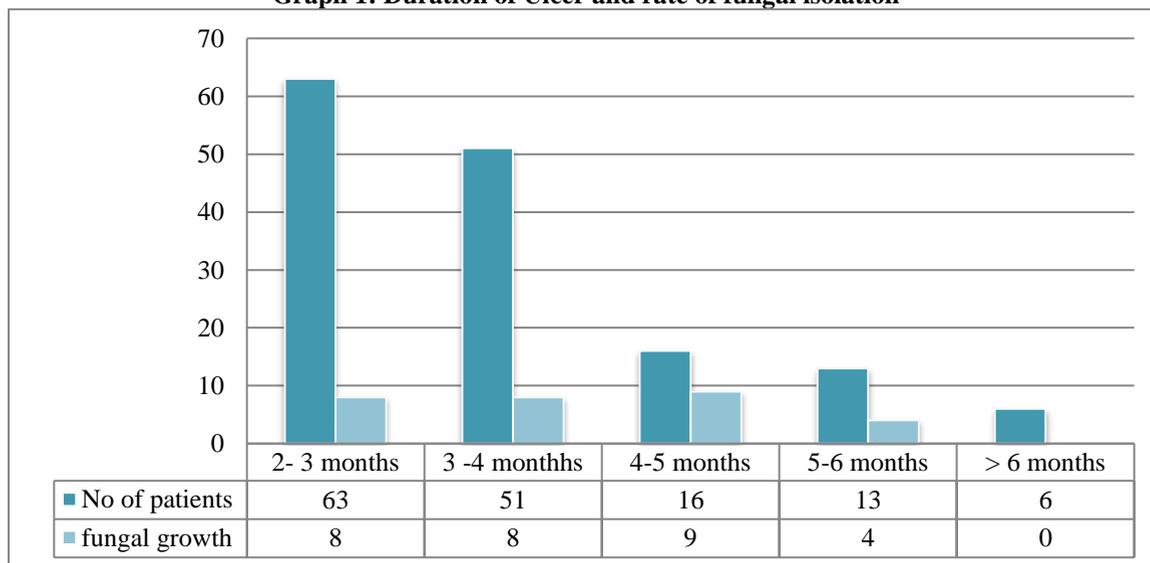
Demographic analysis was as follows:

Age	Patients between 35-85 yrs were included in study. Maximum in the age group of 51-60yrs -32.6 % (49/150). 31-40yrs (14/150), 41-50yrs (31/150), 61-70yrs (38/150), 71-80yrs (18/150).
Gender distribution	Males 66.7 % (100/150) Females 33.3 % (50/150)
Duration of Diabetes	<5 yrs -63 patients, >5 yrs – 87 patients.
Cause of ulcer	Spontaneous -57 % (86/150) Secondary to trauma- 43 % (64/150)
Duration of ulcer	2 to 8 months Maximum 42 % (63/150) between 2 to 3 months depicted in Graph 1
Wagner grading	I-V 43% (64/150) were grade III, 39% (58/150) grade II, 19% (28/150) grade IV.
Co-morbid conditions	Hypertension was the most common co-morbid condition 34.6 % (52/150) others were chronic renal failure, Ischemic heart disease and hypothyroidism.

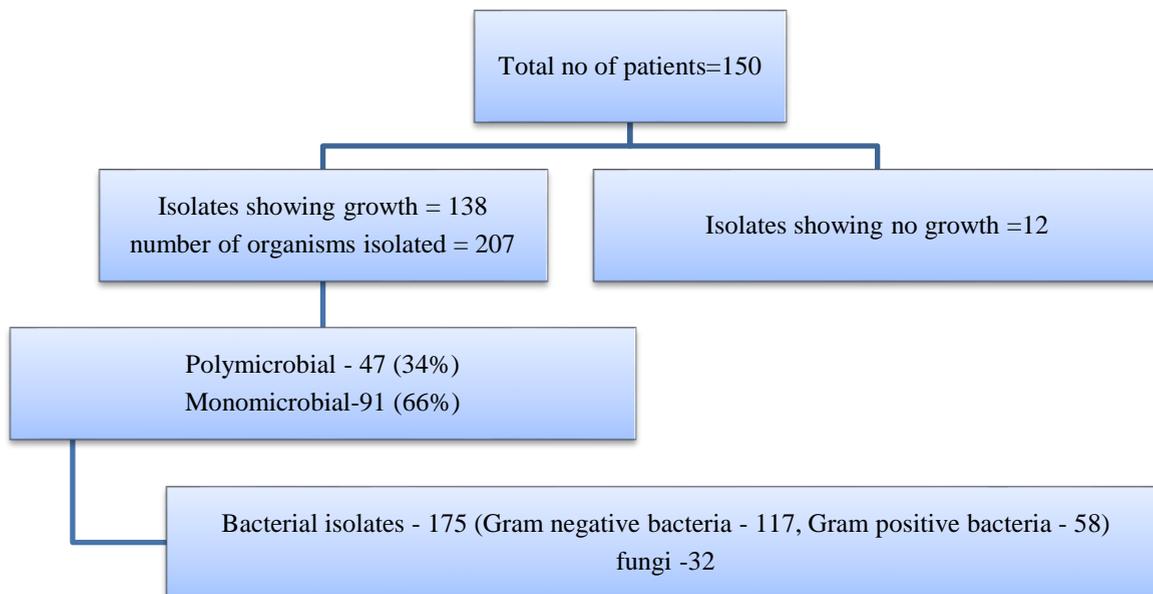
Table 1: Wagner grading

Grade	Lesion
0	Without open lesions, with or without deformity or cellulitis
1	Superficial shallow diabetic ulcer (partial or full thickness)
2	Ulcer extended to ligament, tendon, joint capsule or deep fascia without abscess or osteomyelitis
3	Deep ulcer with abscess, osteomyelitis or septic joint.
4	Partial gangrene
5	Gangrene of entire foot

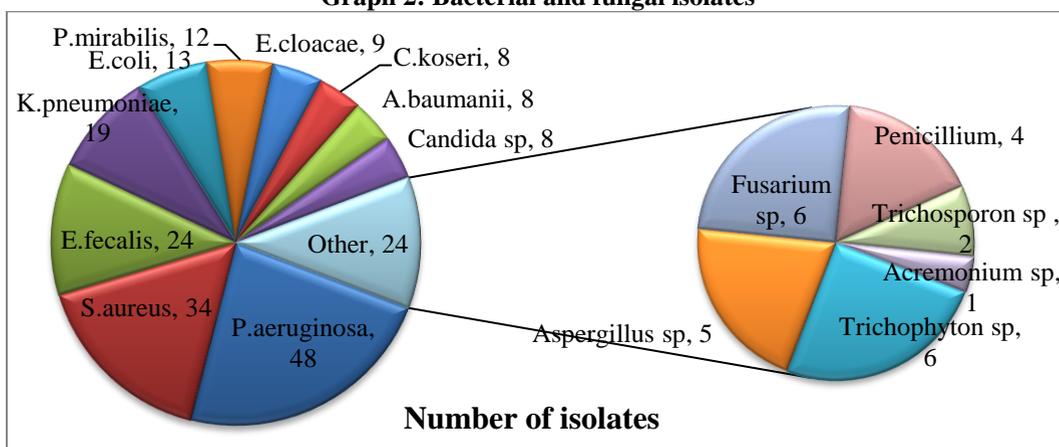
Graph 1: Duration of Ulcer and rate of fungal isolation



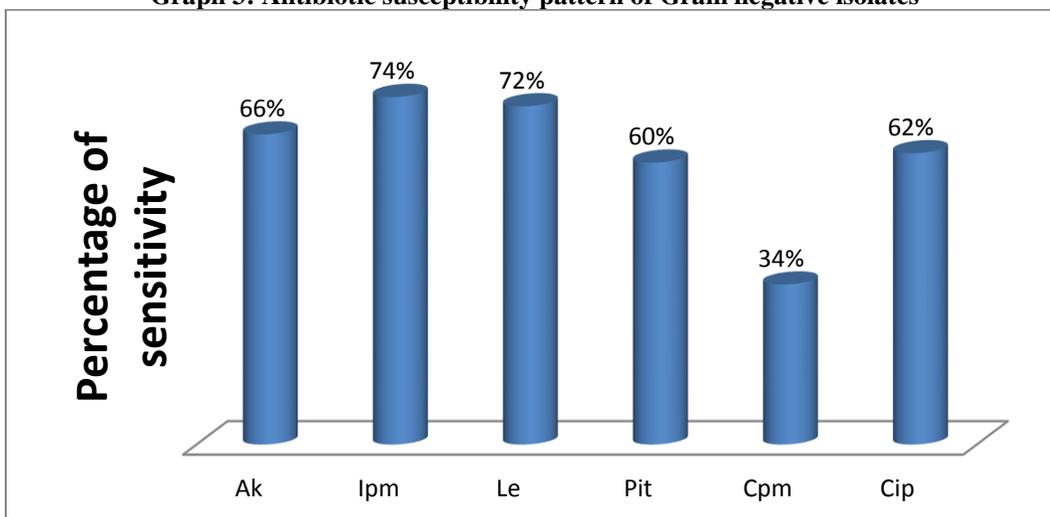
Microbiological analysis of the ulcers: species isolated as depicted in Graph 2:



Graph 2: Bacterial and fungal isolates

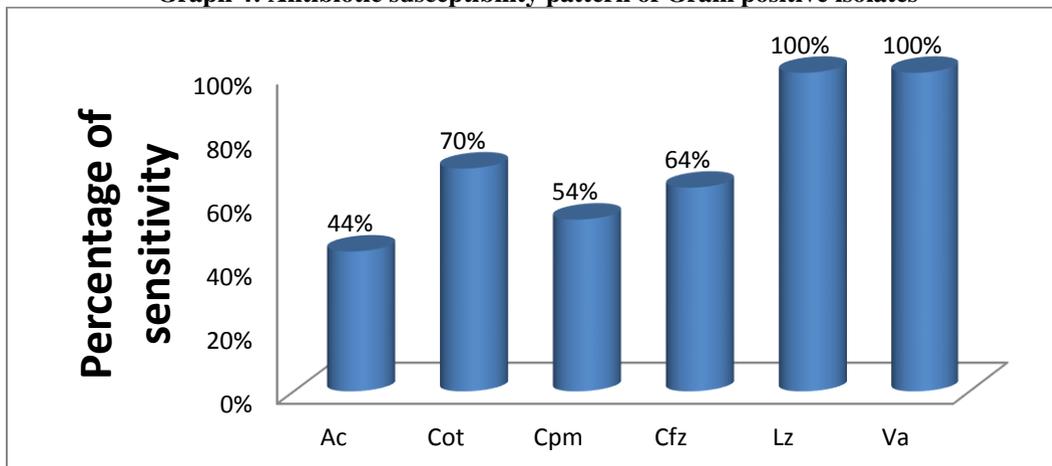


Graph 3: Antibiotic susceptibility pattern of Gram negative isolates



Ak- amikacin, Ipm- imipenem, Le- levofloxacin, Pit- piperacillin tazobactam, Cpm- cefepime, Cip- ciprofloxacin.

Graph 4: Antibiotic susceptibility pattern of Gram positive isolates



Ac- amoxicillin-clavulanic acid, Cot- co-trimoxazole, Cpm- Cefepime, Cfz- cefoxitin, Cd- clindamycin, Lz- linezolid, Va- vancomycin

Pseudomonas aeruginosa was the most common gram negative bacterial isolate accounting for 27.4% (48/175), *Staphylococcus aureus* 19.4% (34/175) was most common gram positive bacteria isolated.

Antibiotic susceptibility pattern of *P. aeruginosa* showed 93% to Piperacillin+ Tazobactam and 74% were susceptible to Imipenem (32% were MBL producers) and only 58% to Cephalosporins. All *S. aureus* isolates were susceptible to Linezolid and Vancomycin and 36% were resistant to Cefoxitin and hence, were considered as Methicillin resistant strains.

In our study, 32 out of 150 patients with diabetic foot ulcers had fungus growth in the tissue specimen obtained from their foot. Ten out of 32 of them were pure fungal growths. The remaining 68.7% (22/32) co-existed with at least one bacteria in the ulcer. *P. aeruginosa* 34% (11/32) was the most common bacterium co-existing with fungal growth in diabetic foot ulcers in our study. The second most common bacterium was *S. aureus* 21.8% (07/32).

Out of 32 fungal isolates, eight *Candida spp* (six- *C. albicans*, one- *C. glabrata* and one- *C. tropicalis*), five *Aspergillus spp* (three- *A. fumigatus*, one- *A. flavus* and one- *A. niger*), six *Trichophyton spp* (four- *T. rubrum* and two- *T. mentagrophytes*), six *Fusarium spp* four *Penicillium spp*, two *Trichosporon spp* and one *Acremonium (Cephalosporium) spp* were identified. Over all *Candida albicans* was the most common fungal isolate 25% (08/32). Fungal positivity was commonly found in ulcers of duration between four - five months which was statistically significant. There was no correlation of duration of ulcer and blood sugar levels of diabetic patients with fungal infection. Significant association was found between fungal infections and ulcers that showed no bacterial growth (*p value* 0.023). Fungal positivity in the study was significantly associated with secondary suturing (Most common), disarticulation/ amputation and Grade IV ulcers (*p value* 0.39).

Statistical analysis was carried out by Chi square / 2x2, 2x3, 3x4 Fisher Exact test and Single proportion Z-test using SPSS software.

Discussion

Diabetes mellitus is a chief health problem, rapidly expanding worldwide. An estimate made in the year 2000 that there were 32 million people with diabetes in India, a number that is predicted to increase to nearly 80 million by 2030.⁽⁶⁾ Infected foot ulcer is a usual cause of morbidity in diabetic patients, ultimately leading to severe complications like gangrene and amputations. There is a 25% chance that a diabetic person might develop a foot ulcer in his life time.⁽⁷⁾ Most of the diabetic foot infections are polymicrobial in nature and mixed organisms are frequently encountered. Emergence of resistance among organisms against the commonly used antibiotics has been evidenced in various studies, the reason being largely due to their indiscriminate use.⁽⁸⁾ Hence, this study has been undertaken in an attempt to understand the demography and microbiological profile of diabetic foot ulcers and aims to identify the most common causative agents and to study their antimicrobial susceptibility pattern.

We studied a total of 150 diabetic subjects with foot ulcers, 66.7% (100/150) of them were males and 33.3% (50/150) females, majority belonging to 51-60 years 32.6% (49/150) similar to study by Raja et al,⁽⁸⁾ Chincholikar et al⁽⁹⁾ and Chaudhry et al.⁽¹⁰⁾ Majority of the subjects affected in these studies also belonged to the same age group, showing that foot complications are common in the elderly. Most of the foot ulcers in our study were of Wagner Grade II 39% (58/150) and Grade III 43% (64/150) similar to a study by Anand et al.⁽¹¹⁾

207 organisms were isolated from 150 clinical samples of diabetic foot ulcers, this represents an

average of 1.38 organisms per ulcer which is lower than that of other studies done by *Raja et al*⁽⁸⁾ and *Bansal et al* which showed an average of 1.52 organisms per ulcer.⁽¹²⁾ The reason could be variation in the condition of patient ulcer and prevalence of pathogenic organisms causing infection from patient to patient.

In our study, polymicrobial growth was seen in 34% (47/138) and monomicrobial in 66% (91/138) cases. Among the bacterial isolates, gram negative bacilli were more commonly isolated than gram positive organisms. *P.aeruginosa* (27.4 %) was the most common gram negative isolate and *S. aureus* (19.4 %) was most common gram positive organism. Similar to a study by *Bansal et al*⁽¹¹⁾ and *Mehta et al*.⁽¹³⁾

As depicted in Graph 3 and 4, most of the gram negative isolates were susceptible to Piperacillin+tazobactam, however there has been an increasing trend in the resistance to Imipenem and Cephalosporins probably due to their irrational use.

36% of the *S. aureus* isolates were MRSA slightly lower compared to study by *Mehta et al*⁽¹³⁾ showing 60% and study by *Bansal et al*⁽¹¹⁾ showing 55% probably due to good infection control practices. 36 % of the isolates were resistant to imipenem and 32 % of them were MBL producers similar to a study by *Kali A et al*.⁽¹⁴⁾

Out of the 150 clinical samples in our study 21% (32/150) were positive for fungal growth, the commonest fungus isolated was *C. albicans* (25%). Similar to a study by *Chincholikar et al* showing 20.8% fungal isolation rate.⁽⁹⁾ Studies in the past from India have reported a much lower rate of isolation (9%) from superficial swabs from diabetic wound patients. However, a recent study has shown a higher prevalence of 27.9% of fungal agents, with majority being *Candida spp* even upto 76.6%.^(15,16)

Fungal growth was seen in long standing ulcers on antibiotic treatment probably because there may be biofilm formation which is one of the most important virulence factors exhibited by *Candida spp*. Biofilms are responsible for persistence of fungal infections and resistance to action of antimicrobials.⁽¹⁵⁾

Conclusion

Antibiotic stewardship and co-ordinated efforts of medical microbiologist and the clinician is required for the adequate management of diabetic foot ulcers and to reduce rise of resistant organisms. Understanding the mechanisms of adhesion and signalling involved in bacterial-fungal interactions may lead to development of better therapeutic modalities. Application of molecular biology-based diagnostic tools would provide better understanding of the wound's ecology. More studies are to be done to assess the role of antifungal agents in diabetic foot wound healing. Reinforcing preventive actions and educating patients about the importance of glycemic control, foot care

would minimize morbidity due to diabetic foot complications.

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