CHAPTER 2
FUNGAL INFECTIONS IN DIABETIC FOOT WOUNDS

Introduction
Diabetes is now a worldwide epidemic (155). India has been one of WHO member country with the highest number of people with diabetes (155). 15% of patients with diabetes develop lower extremity ulcers during their lifetimes. Diabetes is the most common cause for non-traumatic amputation of lower extremities (4, 162). 85% of these lower limb amputations are preceded by polymicrobial infections of the wound (96, 110, 151). Despite proper surgical and antibacterial therapy for infected DLWs, the global long-term outcome of patients was found to be poor; only <50% of these patients had Global Therapeutic Success (74).

Fungal infections among immuno-compromised patients is one of the major health concerns worldwide (35, 57, 74, 80), but the spectrum of fungi infecting DLWs and its pathogenicity have not yet been studied thoroughly. Therefore, clinicians and surgeons treating diabetic foot wounds suspect only the bacterial infections and treat with antibacterial agents. They do not routinely send deep tissue from the wound bed for fungal culture and sensitivity either due to lack of literature support or due to the assumption that there would not be any fungal infections in the DLWs. Surprisingly, our retrospective pilot study showed 27.9% positive fungal culture in 318 diabetic patients with DLWs. We speculate that opportunistic fungi may invade deep into the wounds and contribute to delayed wound healing in some of the diabetic patients who are otherwise immuno-compromised when compared to non-diabetics. The magnitude of fungal infections in diabetic lower extremity in India has been previously studied in limited number of patients.

Aims: To estimate the prevalence of fungi in DLWs and also describe the spectrum of these fungal infections.
Materials and Methods:

Sample size estimation: As there were no available studies on prevalence rate of fungal infections in deep tissue of DLWs, we used our pilot study results for estimation of the prevalence rate of fungal infections in wounds infected diabetic cases. Considering 95% confidence interval and 15% allowable error, the sample size (n) was calculated as 500.

Study population: All type 2 diabetic patients (irrespective of age and sex) who were hospitalized for surgical management of lower extremity wounds from January 2008 on were considered for the study. Their informed consent was obtained and demographic details, duration of lower limb lesion, duration of diabetes, wound assessment based on University of Texas System of Wound Classification was documented. Glycosylated hemoglobin level (HbA1c) by HPLC method, ankle brachial index (ABI), vibration perception threshold (VPT) and transcutaneous oxygen tension (TcPO2) were measured (163). The patients with a history of malignancy, chemotherapy, radiotherapy, or on steroids or antifungal drugs (local or systemic) were excluded from the study. A deep tissue specimen was obtained from the wounds during surgery and sent for fungal and bacterial cultures.

Specimen collection: The slough and necrotic tissue over the wound was surgically debrided in the operation theater. After a thorough wash of the wound with normal saline, a deep tissue specimen of approximately 0.5 X 0.5cm size was taken from the wound bed. The specimen was collected in a sterile container and the tissue was soaked with normal saline. This was transported to our Microbiology lab within 10-15 minutes for further processing.

Fungal culture and sensitivity: The specimen was processed in biological safety cabinet type IIB. The tissue was sliced into tiny fragments (about 1mm cubes) with a sterile scalpel blade. These fragments were placed directly into two Sabouraud’s Dextrose Agar with Chloramphenicol slants, and submerged slightly beneath the surface by using an inoculating needle. These slants were incubated at 30°C and 35°C and observed for 4 weeks. KOH (10%) and Gram stain examination was performed and the results were documented. Fungal species were identified morphologically (48,
and using ID32C strips (miniAPI, bioMerieux, USA) (29, 46, 102, 119). In addition, Corn meal agar morphological study, germ tube and urease tests were also performed for identification of the yeast species. Aspergillus species and other filamentous fungi were identified by slide culture on Potato dextrose agar (PDA) with Lactophenol cotton blue staining.

Antifungal susceptibility test for yeast was done with ATB Fungus-3 strips (miniAPI, bioMerieux, USA) (129). MIC of <0.125 mcg/ml for itraconazole, <1.0 mcg/ml for voriconazole, < 8.0 mcg/ml for fluconazole and < 4.0 mcg/ml for flucytosine was considered as sensitive. For amphotericin b, MIC values were not detected by machine. Standard strains, C.albicans ATCC 90028, C.tropicalis ATCC 750, C.parapsilosis ATCC 22019, C.krusei ATCC 6258 were used as controls in the study. The susceptibility for filamentous fungi was not performed.

**Bacterial culture**: Part of the sterile deep tissue specimen was crushed or ground in sterile mortar and pestle in Biosafety cabinet. Gram staining was done and the crushed specimen was inoculated in Thioglycollate medium. The sample was streaked on the Sheep Blood Agar (SBA) and MacConkey Agar (MA). The SBA was kept in 5% CO₂ incubator; and MA and Thioglycollate in O₂ atmosphere at 37°C incubator. Bacterial isolates were identified by standard biochemical tests and susceptibility testing was performed as per CLSI guidelines.

**Analysis**: Data was recorded in SPSS software (version: 11). The percentage of fungal and bacterial isolates was computed by applying descriptive statistics. To test the co-existence of fungi and bacteria; and the statistical association of fungal infection with wound depth, Chi-Square test was applied. Student’s t-test was applied to test the statistical significance of the difference in mean values of parameters such as age, duration of foot lesion, duration of diabetes, HbA1c, ABI, VPT and TcPO₂ between the two groups (presence and absence of fungi).

**Results**
Of 518 patients, 382 (73.7%) were males and 136 (26.3%) were females. The mean age of the study population was 60.8 +/-10.2 years, duration of diabetes was 193.4 +/-
97.3 months, duration of lower limb lesion was 43.7 +/- 72.8 days, HbA1c was 9.8 +/- 2.4 %, ABI was 1.02 +/- 0.51, VPT 43.7 +/- 9.6 volts and TcPO2 was 33.1 +/- 16.4 mm Hg.

Prevalence of fungi in deep tissue of diabetic lower limb wounds was 27.2% (141/518 patients). Among the isolates, 76.6% (108/141) were Candida species, 12.8% (18/141) were Trichosporon species, 8.5% (12/141) were filamentous fungi and other yeast were 2.1% (3/141) (Table 2.1). The predominant species were Candida parapsilosis (25.5%), Candida tropicalis (22.7%), Trichosporon asahii (12.8%) and Candida albicans (10.6%), Aspergillus species (5.0%).

**Table 2.1. Spectrum of Fungi Isolated from Deep Tissue of Diabetic Foot Wounds.**

<table>
<thead>
<tr>
<th>Sl. No</th>
<th>Species</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Candida parapsilosis</td>
<td>36</td>
<td>25.5</td>
</tr>
<tr>
<td>2</td>
<td>C. tropicalis</td>
<td>32</td>
<td>22.7</td>
</tr>
<tr>
<td>3</td>
<td>T. asahii</td>
<td>18</td>
<td>12.8</td>
</tr>
<tr>
<td>4</td>
<td>C. albicans</td>
<td>15</td>
<td>10.6</td>
</tr>
<tr>
<td>5</td>
<td>Aspergillus sp</td>
<td>7</td>
<td>5.0</td>
</tr>
<tr>
<td>6</td>
<td>C. guilliermondii</td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td>7</td>
<td>C. non-albicans</td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td>8</td>
<td>C. glabrata</td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td>9</td>
<td>Fusarium sp</td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td>10</td>
<td>C. sake</td>
<td>4</td>
<td>2.8</td>
</tr>
<tr>
<td>11</td>
<td>Zygosaccharomyces sp</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>12</td>
<td>Kodamaea ohmeri</td>
<td>3</td>
<td>2.1</td>
</tr>
<tr>
<td>13</td>
<td>C. globosa</td>
<td>2</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>C. krusei</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>15</td>
<td>Penicillium sp</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>16</td>
<td>C. lusitaniae</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>17</td>
<td>C. famata</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td>18</td>
<td>C. melibiosa</td>
<td>1</td>
<td>0.7</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>141</strong></td>
<td><strong>100.0</strong></td>
</tr>
</tbody>
</table>
Sensitivity to fluconazole (FLZ), itraconazole (ITR), voriconazole (VOR), flucytosine (FLCYT) and amphotericin b (AMPB) was tested for 130 yeast isolates. The resistance rate of the fungal isolates was 1.5% (2/130) for FLCYT, 3.9% (5/130) for FLZ, 6.9% (9/130) for AMPB, 6.9% (9/130) for VOR, and 17.7% (23/130) for ITR. Of the 32 Candida tropicalis and 15 Candida albicans isolates 3 of the former and 2 of the latter were resistant to FLZ. One each of the 32 Candida tropicalis and 18 Trichosporon asahii isolates were resistant to FLCYT. Similarly, resistance to AMPB was seen in 3 cases of Candida parapsilosis, 2 cases of Zygosaccharomyces species and one each of Candida tropicalis, Trichosporon asahii, Candida guillermondii and Candida lusitaniae. VOR resistance was seen in Candida tropicalis (3/32), Trichosporon asahii (1/18), Candida albicans (2/15), Candida glabrata (2/4) and Zygosaccharomyces species (1/3). Candida tropicalis showed high incidence of resistance to ITR (9/32).

An analysis was performed to look for patients who had purely bacterial or fungal infections or mixed bacterial and fungal infections, or neither bacteria nor fungi in DLWs. It was found that 5.8% (30/518) of these patients had only fungal infections, while 58.3% (302/518) patients had only bacterial infections, whereas 14.5% (75/518) patients had neither fungal nor bacterial infections, and 21.4% (111/518) had both bacteria and fungi in their deep tissue (Fig. 2.1).

![Figure 2.1: Microbial flora in deep tissue of diabetic lower limb wounds](image-url)
The prevalence of bacteria in deep tissues of DLWs was 79.7% (413/518). About 607 bacterial isolates were cultured from 413 patients, hence the isolation rate was 1.5 (607/413). Among these, 55.3% were gram negative and 44.7% were gram positive. The predominant bacteria cultured were Enterococcus faecalis (14.1%), Staphylococcus aureus (12.2%), Pseudomonas aeruginosa (10.8%), Klebsiella pneumoniae (7.9%), Escherichia coli (7.7%) and Coagulase negative Staphylococci (5.8%) (Table 2.2).

Table 2.2. Spectrum of bacteria isolated from deep tissue of diabetic lower extremity wounds (n = 518).

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Species</th>
<th>Frequency</th>
<th>Percentage</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Enterococcus faecalis</td>
<td>73</td>
<td>14.1</td>
</tr>
<tr>
<td>2</td>
<td>Staphylococcus aureus</td>
<td>63</td>
<td>12.2</td>
</tr>
<tr>
<td>3</td>
<td>Pseudomonas aeruginosa</td>
<td>60</td>
<td>10.8</td>
</tr>
<tr>
<td>4</td>
<td>Klebsiella pneumonia</td>
<td>41</td>
<td>7.9</td>
</tr>
<tr>
<td>5</td>
<td>E.coli</td>
<td>40</td>
<td>7.7</td>
</tr>
<tr>
<td>6</td>
<td>Coagulase negative Staphylococcus sp</td>
<td>30</td>
<td>5.8</td>
</tr>
<tr>
<td>7</td>
<td>Nonfermenter Gram Negative Bacilli</td>
<td>24</td>
<td>4.7</td>
</tr>
<tr>
<td>8</td>
<td>Enterobacter sp</td>
<td>16</td>
<td>3.1</td>
</tr>
<tr>
<td>9</td>
<td>Beta hemolytic streptococcus</td>
<td>15</td>
<td>2.9</td>
</tr>
<tr>
<td>10</td>
<td>Proteus mirabilis</td>
<td>10</td>
<td>1.9</td>
</tr>
<tr>
<td>11</td>
<td>Proteus vulgaris</td>
<td>10</td>
<td>1.9</td>
</tr>
<tr>
<td>12</td>
<td>Streptococcus sp</td>
<td>8</td>
<td>1.5</td>
</tr>
<tr>
<td>13</td>
<td>Citrobacter freundii</td>
<td>7</td>
<td>1.4</td>
</tr>
<tr>
<td>14</td>
<td>MDR Pseudomonas aeruginosa</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>15</td>
<td>MRSA</td>
<td>4</td>
<td>0.8</td>
</tr>
<tr>
<td>16</td>
<td>Diphtheroid sp</td>
<td>3</td>
<td>0.6</td>
</tr>
<tr>
<td>17</td>
<td>Citrobacter diversus</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>18</td>
<td>Serratia sp</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>19</td>
<td>Gram Positive Bacilli</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>20</td>
<td>MDR E. coli</td>
<td>2</td>
<td>0.4</td>
</tr>
<tr>
<td>21</td>
<td>Morganella morgani</td>
<td>1</td>
<td>0.2</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td></td>
<td><strong>413</strong></td>
<td><strong>79.7</strong></td>
</tr>
</tbody>
</table>
Among patients who had bacterial infections, 47.5% (246/518) had only single bacterial colonization in the deep tissue, while 27.0% (140/518) patient had two different types of bacteria co-existing in the wound and 5.2% (27/518) had three types of bacteria in the deep tissue (Table 2.3).

Table 2.3. Coexistence of fungal and bacterial infections in deep tissues of diabetic lower limb wound.

<table>
<thead>
<tr>
<th>Bacteria isolated from deep tissue</th>
<th>Positive fungal cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>One isolate (n=246)</td>
<td>57</td>
</tr>
<tr>
<td>Two isolates (n=140)</td>
<td>46</td>
</tr>
<tr>
<td>Three isolates (n=27)</td>
<td>8</td>
</tr>
<tr>
<td>Total (n= 413)</td>
<td>111</td>
</tr>
</tbody>
</table>

p= 0.12

About 20.3% (105/518) of the total patients had no bacterial growth in the deep tissue. The presence of co-existent fungal infection was the same in patients who had one, two or three bacterial species isolated (p = 0.12). Among the bacterial isolates, the predominant isolates were gram negative (70.46%). On analysis, we found no significant correlation between fungal infection and gram positive or gram negative bacteria (p= 0.81) isolated from the deep tissue.

On analyzing the depth of DLWs of the study population, we found that 40.3% (209/518) patients had a wound extending up to muscles, 45.4% (235/518) patients, up to the tendon or capsule and 14.3% of patients up to the adjacent joint or bone (Table 2.4). Though fungal isolates were more in grade 2 wounds as compared to grade 1 or grade 3, it was not statistically significant (p= 0.59).
Table 2.4. Fungal infections and the depth of diabetic foot lesions.

<table>
<thead>
<tr>
<th>Wound Grade*</th>
<th>Presence of fungi</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.</td>
</tr>
<tr>
<td>II (Muscle only) (n=209)</td>
<td>54</td>
</tr>
<tr>
<td>III (Tendon and capsule) (n=235)</td>
<td>69</td>
</tr>
<tr>
<td>IV (Joint and bone) (n=74)</td>
<td>18</td>
</tr>
<tr>
<td>Total (n=518)</td>
<td>141</td>
</tr>
</tbody>
</table>

p=0.59 * University of Texas Wound classification

Fungal infection was significantly associated with glycosylated hemoglobin level (p = 0.04) of the patients, but not with the age, sex, duration of diabetes, duration of foot lesion, ABI, VPT or TcPO2 (Table 2.5).

Table 2.5. Comparing deep tissue fungal infection with age, duration of diabetes, glycemic status, duration of lesion and neurovascular status of the lower limb of the patients with diabetic lower limb wounds.

<table>
<thead>
<tr>
<th>Fungi</th>
<th>Agea</th>
<th>Woundb</th>
<th>HbA1cc</th>
<th>ABId</th>
<th>VPTe</th>
<th>TcPO2f</th>
<th>DMg</th>
</tr>
</thead>
<tbody>
<tr>
<td>No</td>
<td>n</td>
<td>377</td>
<td>364</td>
<td>152</td>
<td>273</td>
<td>279</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>60.6</td>
<td>41.2</td>
<td>9.5</td>
<td>1.0</td>
<td>43.6</td>
<td>33.1</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>10.0</td>
<td>73.0</td>
<td>2.4</td>
<td>0.57</td>
<td>9.3</td>
<td>16.1</td>
</tr>
<tr>
<td>Yes</td>
<td>n</td>
<td>141</td>
<td>136</td>
<td>58</td>
<td>102</td>
<td>107</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>mean</td>
<td>61.2</td>
<td>50.2</td>
<td>10.3</td>
<td>0.9</td>
<td>43.9</td>
<td>32.8</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>10.8</td>
<td>71.7</td>
<td>2.2</td>
<td>0.3</td>
<td>10.1</td>
<td>17.0</td>
</tr>
<tr>
<td>p value</td>
<td>0.57</td>
<td>0.22</td>
<td>0.04</td>
<td>0.35</td>
<td>0.80</td>
<td>0.86</td>
<td>0.30</td>
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</table>

<table>
<thead>
<tr>
<th>Total</th>
<th>n</th>
<th>518</th>
<th>500</th>
<th>210</th>
<th>375</th>
<th>386</th>
<th>310</th>
<th>375</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>mean</td>
<td>60.7</td>
<td>43.6</td>
<td>9.7</td>
<td>1.0</td>
<td>43.7</td>
<td>33.0</td>
<td>193.2</td>
</tr>
<tr>
<td></td>
<td>SD</td>
<td>10.2</td>
<td>72.7</td>
<td>2.3</td>
<td>0.5</td>
<td>9.5</td>
<td>16.3</td>
<td>97.3</td>
</tr>
</tbody>
</table>

*a Age in years, b Wound duration in days, c Glycosylated hemoglobin in percentage, d Ankle Brachial Index (ratio), e Vibration perception threshold in volts, f Transcutaneous partial pressure of oxygen in mm Hg, g Duration of diabetes in months.
Discussions
The incidence of diabetes is increasing rapidly. Diabetic foot ulcers affect millions of people worldwide and impose tremendous medical, psychosocial and financial loss or burden. Patient care for diabetic foot ulceration is complex and necessitates multi-professional collaboration to provide comprehensive wound care (162). 85% of the lower limb amputations in diabetics are preceded by polymicrobial infections of the wounds (87, 88). Many studies have been done on the prevalence and spectrum of bacterial infections, the role of systemic/local antibiotics and their effect on wound healing. However, the magnitude of fungal infections in diabetic foot wounds is an area which has received very little attention. Studies have shown that toe web dermatophyte infection provides a hospitable niche for subsequent colonization by bacteria. Exacerbation of a mild dermatophyte infection (dermatophytosis simplex) can arise in the occlusive environment of the toe web space. Fungal infection induces damage to the stratum corneum, which allows overgrowth of resident bacteria and maceration, itching, and often malodor at the site (71, 83).

Mlinaric Missoni et al from Croatia had reported fungal incidence in tissue biopsies of 22 diabetic patients who had clinical evidence of fungal infections (56, 99). The predominant isolates were C. parapsilosis (45.5%), C. tropicalis (22.7%), C. albicans (9.1%) and C. glabrata (9.1%). Bansal et al from India had reported 9% isolation of fungi from superficial swabs taken from 103 patients with diabetic foot wounds (17). Predominant species were C. tropicalis (29%), C. albicans (14%) and C. guillermondii (7%); followed by Aspergillus flavus (21%), Aspergillus niger (14%) and Fusarium sp. (14%) were the common isolates. The same spectrum of fungi was isolated from immune-compromised patients’ blood by Pfaller et al and Bedeni et al (20, 113). Though these studies confirm pathogenic fungal infections in DLWs, the spectrum of fungi and its prevalence has not been explored properly in deep tissue of the wounds.

Our study shows high prevalence and a wide spectrum of fungi (18 different species) in deep tissues of DLWs when compared to the previous studies. Among these, 89.4% are yeasts and 10.6% are filamentous fungi. The isolates obtained by us from deep tissue of DLWs were similar to the spectrum of species isolated from blood streams by Gonzalez et al (48). C. parapsilosis emerged as the most common fungal isolate in
our study. Studies have reported that *C. parapsilosis* has dramatically increased in significance and prevalence over past two decades and is known to be one of the leading causes for invasive *Candidal* disease (140). *Aspergillus* infections are to be considered as the differential diagnosis of slowly progressive destructive wound infections(57). It has been found as the most common filamentous fungi isolated from DLWs of our patients.

Sensitivity to FLZ, ITR, VOR, FLCYT and AMPB was tested for 130 yeast isolates. In-vitro susceptibility was highest to FLCYT and lowest to ITR for these isolates. We observed intra and inter species variations in susceptibility to antifungal agents. Similar results were reported by Cornelia Las-Florl et al (78). In their study, AMPB and posaconazole were found to be active against most of the pathogens including species that cause rare and difficult-to-treat infections. In our study, about 6.9% of the yeasts showed resistance to AMPB. *C. parapsilosis* and *Zygosaccharomyces* species were the most common species showing resistance to AMPB. We did not ask patients for their past exposure to AMPB. This might have provided an insight into the cause for resistance. Resistance to FLZ was detected to be low (3.9%) in our study which is consistent with Thean et al’s study. In their study the resistance was observed in 3.2% of all *Candida* infections in the bloodstream (138). In the same study, about 37.5% of the fungal isolates were resistant to ITR, whereas it was only 17.7% in our study. In our study, resistance of *Candida tropicalis* to FLZ was 6.25%, whereas it was 2.7% in Thean et al’s study. Though there have been reports on itraconazole-resistant and fluconazole-susceptible isolates of *Candida* species, these remain unconfirmed (159). Further studies are required in this regard. With the past series of studies, it is observed that the susceptibility of *Candida* species to antifungal agents varies over time and among the countries and regions (112). Case to case treatment with culture specific antifungal therapy would provide greater benefit than presumptive therapy.

Chronic wounds have a complex microbiological environment with a mixed flora that changes over time (77). Coagulase negative *Staphylococci*, *Streptococcus* sp, *Corynebacterium* sp and *Staphylococcus aureus* populate the wound initially before facultative anaerobic gram-negative bacilli, such as *E. coli*, *Klebsiella*, or *Proteus* sp take up residence, usually days or even weeks later. The longer an ulcer remains
unhealed, the more likely it is that it will acquire multiple aerobic organisms, as well as a significant anaerobic population. Chronic wounds have a statistically higher proportion of anaerobes as compared to acute wounds (77). Scot E et al found 30% of anaerobes in diabetic wounds whereas it was 62% in pressure wounds (127). Our study points to the fact the deep tissue of the DLWs must be cultured for fungi and bacteria as diabetic patients may present with sterile wounds; or have purely bacterial or fungal infections; or mixed bacterial and fungal infections. This raises interesting questions about the contribution of these deep-tissue fungal infections to the delay in the healing of DLWs especially in those few patients that had only fungal infections. The bacteria that we isolated from DLWs were predominantly gram negative which was consistent with Lipsky et al’s (87) and Diane et al’s studies (38).

Some bacteria work together in microbial synergy. In mixed aerobic/anaerobic infections, microbial synergy frequently exists. The effect of synergy between two bacteria can be devastating for the host, especially if the synergy fosters a rapidly destructive necrotizing fasciitis. Less invasive microorganisms like coliforms can be synergistic with more virulent ones and play a crucial role in wound infection (77). The synergy between bacteria and fungi may have a role in wound healing. Studies have shown that when two opportunistic pathogens, P. aeruginosa and C.albicans are found together, the former forms a dense biofilm on the filaments of the latter and kills the fungus (37). We also observed the same in our study. Of the 15 C.albicans that we isolated from DLWs none were found in patients who had P. aeruginosa infection in their wounds. 2/15 C. albicans isolates were found in the presence of Staphylococcus aureus in our study. Preliminary studies have shown that Staphylococcus aureus and C.albicans, appears to be initially synergistic (132). Further work is definitely warranted to prove synergy of fungi/bacteria in chronic wounds.

When compared to PCR or bTEFAP (bacterial Tag Encoded FLX Amplicon Pyrosequencing), culture-based methods were found insufficient for characterizing complex polymicrobial communities in chronic wounds (76, 127, 128). By using molecular methods, a wide-range of bacteria including fastidious anaerobic bacteria in chronic wounds were identified that were not observed using culture-based methods (76).
The limitation of our study is that we used only classical methods and not molecular methods to identify fungi and bacteria from DLWs. We also did not do susceptibility test for filamentous fungi and culture for anaerobic bacteria.

In summary, our study revealed that there is a high prevalence of fungal infection in deep tissues of diabetic lower extremity wounds. About one in four diabetic patients with lower limb wounds harbored a deep tissue fungal infection. These fungi were found to infect wounds either alone or in conjunction with bacteria. Patients with poor glycemic control had significantly higher fungal infection, but no statistically significant association of fungal infections was observed with patient’s age, sex, ABI, VPT, TcPO2, depth of the wound, and duration of diabetes and limb lesion. Similar finding was observed by Gadepalli R et al, wherein the multi-drug resistant bacterial organisms (MDROs) showed significant association with poor glycemic control (122). Most of the yeast isolates were susceptible to FLCYT and FLZ when compared to the other antifungal agents tested.

More research on evaluating, studying, and treating chronic DLW pathogenic biofilms are required. Application of molecular based diagnostic tools would provide better understanding of the wound’s ecology and will allow clinicians to better manage the wounds and improving the prognosis of the patient. Understanding the mechanisms of adhesion and signaling involved in bacterial-fungal interactions may lead to develop novel therapeutic strategies for chronic DLWs. More studies are to be done to assess the role of antifungal agents in diabetic foot wound healing. Diabetic foot infections require a careful attention and coordinated management of a multidisciplinary foot-care team, which includes an infectious disease specialist and a medical microbiologist. Reinforcing preventive actions and educating patients about the importance of glycemic control, using appropriate footwear at all times, avoiding foot trauma, daily self-examination of foot and early reporting to the health professional for any change over the foot would minimized morbidity and mortality due to diabetic foot complications.