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ORIGINAL ARTICLE

Spectrum of microorganisms identified in diabetic foot ulcers; Latest trends in underdeveloped countries.

Syeda Hina Abbas[1], Syeda Shaheera Zaidi[2], Nabiya Sandeelo[3], Sambreen Zameer[4], Shazia Nazar[5], Syed Hussain Azhar Rizvi[6]

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ABSTRACT… Objective: To see the common microorganisms infecting diabetic foot ulcers in a tertiary care hospital of Sindh, Pakistan. **Study Design:** Cross Sectional Descriptive study. **Setting:** Department of Medicine Civil Hospital, Karachi. **Period:** June 2023 to November 2023. **Methods:** It included 102 patients of all ages and gender, who consented to participate and presented with diabetic foot ulcer. Their demographic data and data of isolated microorganism obtained from wound were recorded and analyzed in SPSS version 24. **Results:** There were 102 patients in our study who visited OPD as case of DFU and consented to take part in study. Mean age was 57.19 ±11.68 Years 24- 90- years patients were included. The highest number of patients 65 (63.7%) were in the 46-65 years age group, followed by the age group of more than 65 years with 21 (20.6%) of the patients. There were 31 (30.4%) females and 71 (69.6%) patients were males. The ratio of male-to-female participants was 2.2:1. Wagner grade of lesion was 40 (39.2%) were in grade 2 followed by 23 (22.5%) grade Mean age of patients who develop infection in their diabetic foot ulcer i.e. DFI was 56.64 ± 11.62 Years 24- 90- years. Patients were included. The highest number of patients 48 (64.4%) were in the 46-65 years age group, followed by the age group of more than 65 years with 17 (22.7%) of the patients. There were 25 (33.3%) females and 50(66.7%) patients were males. Mean duration of diabetes was 3.83 ±1. Wagner grade of lesion was 27(36%) were in grade 2 followed by grade 3 and 4 which was 18(24%) each. Among the 75 cases from which pathogens were isolated 46(61.3%) were monomicrobial and 29(38.7%) were Polymicrobial. Based on gram staining, 70.67% were gram negative isolates 18.67%were gram positive isolates and 10.67% mixed gram positive and negative isolates. Overall, pseudomonas auraginosa was commonest microbe isolated (41.3%) followed by Klebsiella which is 17.33%. MRSA was commonest among gram positive organism isolated from wound. **Conclusion:** Diabetic foot ulcers predominantly get infected by Gram negative bacteria. Proper hygienic practices must be encouraged. Appropriate educational programs targeting awareness for diabetes and related complications must be developed to prevent DFU in this cohort of population.

Key words: Diabetes, Diabetic Foot Ulcer, Diabetic Foot Infection.

INTRODUCTION

Diabetes is a major global health issue, with continuously rising prevalence worldwide, which is estimated to be 592 million by 2035.[1] Based on rising prevalence of the diseases in Pakistan, IDF ranked us among top 10 countries. Prevalence was 8.7% as per survey of 1994 upswings to 26.3% in 2016 survey.[2] Situation is undoubtedly alarming for socioeconomically poor country like Pakistan. Poorly managed diabetes brings a myriad of concerns, predisposing a patient to develop several complications which are lethal and devastating thus increasing disease related morbidity and mortality, worsening quality of life and increasing economic burden on patients.[3] Being a multi system disease with multiple complications, diabetes is challenging for health practitioners from a variety of specializations.[4] Foot care is specifically important in diabetic patients. Diabetic patients already have altered immunity, peripheral neuropathy and ischemia, thus prone to develop DFU in case of even minor trauma. Poorly managed diabetes increase the likelihood of developing Diabetic foot ulcer (DFU). 19% to 34% of diabetics are predicted to experience a DFU at some point in their lives.[5] The expenses of providing care for DFU patients are high. Ulcer can remain superficial

1. FCPS, Assistant Professor Pathology, Dow University of Health Sciences, Karachi.
2. FCPS, Assistant Professor Medicine, Dow University of Health Sciences, Karachi.
3. FCPS, Assistant Professor Medicine, Dow University of Health Sciences, Karachi.
4. M.Phil, Associate Professor Pathology, Dow University of Health Sciences, Karachi.
5. M.Phil, Ph.D, Associate Professor Physiology, Dow University of Health Sciences, Karachi.
6. FCPS, Assistant Professor Medicine, Dow University of Health Sciences, Karachi.

Correspondence Address:
Dr. Syeda Hina Abbas
Department of Pathology
Dow University of Health Sciences, Karachi.
hina.abbas@duhs.edu.pk

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but has a potential to complicate by developing osteomyelitis.[6] Poorly perfused, neuropathic, traumatic foot ulcer offers a nidus and enriched setting for growth of microbes, become infected, and termed as diabetic foot infection (DFI). These infections can be challenging to treat.[7] Even after repeated cycles of antibiotics, there may still be little chance of a clinical resolution and lesion is likely to end up in most feared consequence of DFI, which is amputation. In order to address this therapeutic dilemma, it is essential to have data of pathogens infecting DFU in our part, with hope to ultimately conquer the disease by emerging novel therapeutic agents.

METHODS

This cross sectional descriptive study was carried out at Department of Medicine Civil hospital Karachi from June 2023 to November 2023 The sample size stands to be n=102 calculated by using Open Epi software, 95% Confidence interval, 5% margin of error and prevalence of diabetic foot ulcer was taken to be 7.02% population of Sindh taken 47.9 million as per data of 2020. Sample comprises of patients of all ages and gender, who consented to participate and presented with diabetic foot ulcer admitted in ward or visiting OPD.

Patients meeting inclusion criteria but not consented for the study, other foot ulcers and foot infection in persons without history of diabetes, Wagner Grade I lesion and Patient with diabetic foot ulcer with coexisting autoimmune or other vascular/neuropathic disorders were excluded from study. Data of age gender, history of onset of diabetes, duration of diabetes and duration of wound along with wegners grade of lesion, outcome and culture results was collected on a preforma which was specifically designed for this study. Ulcers were graded according to Wagner Grading System which grades intact skin as 0 superficial ulcer as 1 while ulcers extending to bone and tendon or capsule graded as 2, deep ulcers with abscess or osteomyelitis as 3, partial foot gangrene as 4 and whole foot gangrene as 5.

Sample collection: pus samples were collected either on the day of admission or OPD visit. All aseptic measures were taken sterile pus swab, labelled with dual identification and was used to collect pus from deepest part of wound in a rotatory manner. Special precautions were taken to prevent swab touching other surfaces.it was immediately covered and sent to microbiology laboratory of the same hospital. Percentages and Frequency for gender, different classes of bacteria were recorded. Mean and standard deviation for age and duration of diabetes was also calculated. Data expressed as mean ± standard deviation (SD) for continuous variables, and as frequency with percentage for categorical variables. We sought ethical approval from Institutional Review Board (IRB) Dow University of Health Sciences (IRB-2959/DUHS/Approval/2023/188) and also obtained written informed consent from all the participants.

RESULTS

There were 102 patients in our study who visited OPD as case of DFU and consented to take part in study. Table-I shows the demographic characteristics of study population. Mean age was 57.19 ±11.68 Years 24- 90- years patients were included. The highest number of patients 65 (63.7%) were in the 46-65 years age group, followed by the age group of more than 65 years with 21 (20.6%) of the patients. There were 30.4% female and 69.9% male patients. Mean duration of diabetes was 3.87±1.17 while Duration of wound was 2.17±0.71.wagner grade of lesion is also shown in table.

Demographic characteristics of patients who develop infection in their diabetic foot ulcer i.e. DFI are shown in Table-II. Mean age was 56.64 ± 11.62 Years 24- 90- years. Patients were included. The highest number of patients 48 (64.4%) were in the 46-65 years age group, followed by the age group of more than 65 years with 17 (22.7%) of the patients.

Among the 75 cases from which pathogens were isolated 46(61.3%) were monomicrobial and 29(38.7%) were polymicrobial (Figure-1).

Age (Years)	
Mean ± SD	57.19 ± 11.68
Range(min-max)	24-90
25-45	16(15.7%)
46-65	65(63.7%)
>65	21(20.6%)
Gender	
male	71(69.6%)
female	31(30.4%)
Duration of diabetes	
Mean ± SD	3.87± 1.17
Range(min-max)	1-5
Duration of wound	
Mean ± SD	2.17 ± 0.71
Range(min-max)	1-3
Wegners Grade of Lesion	
Grade 2	40(39.2%)
Grade 3	21(20.6%)
Grade 4	23(22.5%)
Grade 5	18(17.6%)

Table-I. Characteristics of patients who presented with DFU

Age (Years)	
Mean ± SD	56.64 ± 11.62
Range(min-max)	24-90
25-45	10(13.3%)
46-65	48(64.4%)
>65	17(22.7%)
Gender	
male	50(66.7%)
female	25(33.3%)
Duration of diabetes(years)	
Mean ± SD	3.83 ±1.256
Range(min-max)	1-5
Wegners grade of lesion	
Grade 2	27(36%)
Grade 3	18(24%)
Grade 4	18(24%)
Grade 5	11(--- %)

Table-II. Characteristics of patients who presented with DFIs

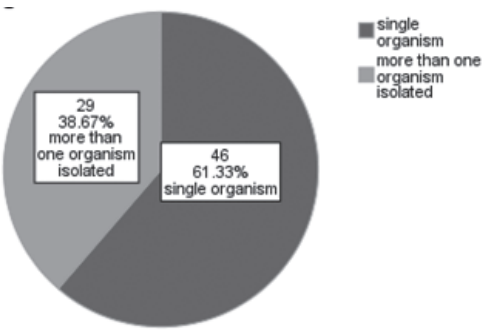


Figure-1

Distribution of organism based on gram stain is shown in figure 6 which exhibits 70.67% gram negative isolates 18.67% gram positive isolates and 10.67% mixed gram positive and negative isolates (Figure-2)

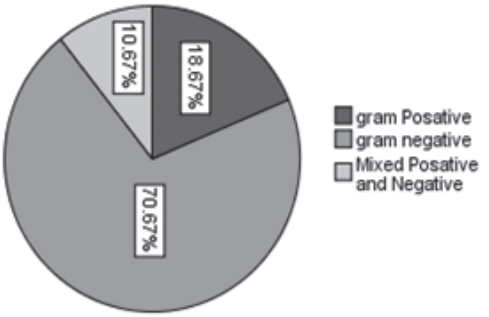


Figure-2

Bacteriological profile of these positive culture isolate is shown in Table-III and frequency of isolated gram negative and gram positive organisms can be seen in Figure-3 and 4 respectively.

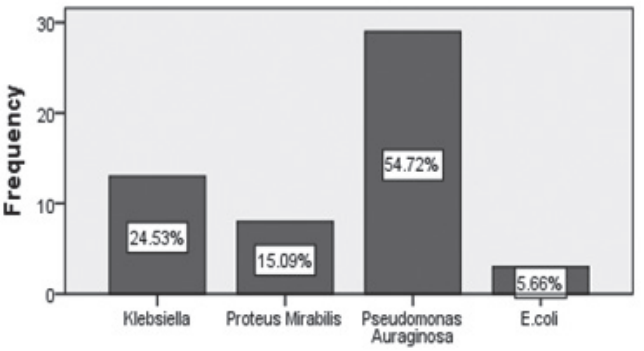


Figure-3

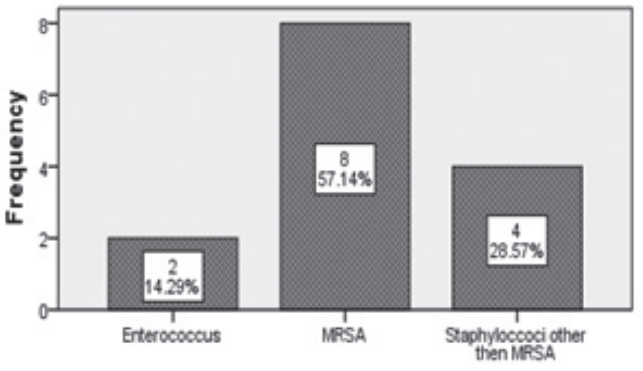
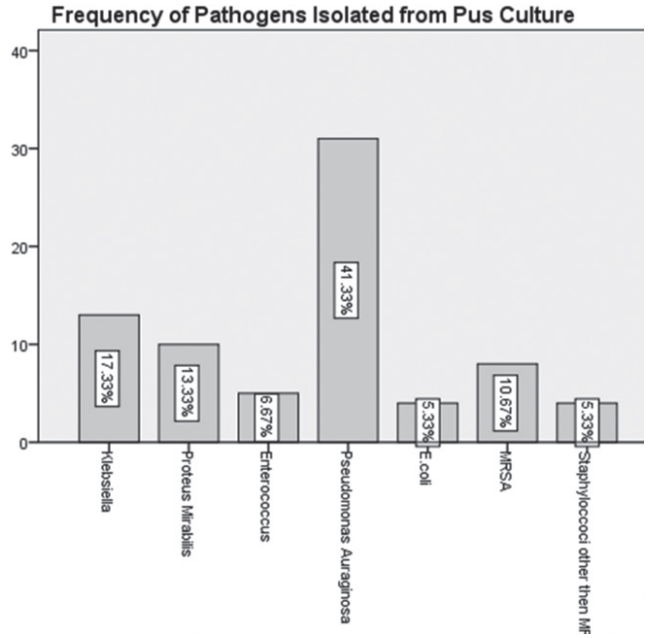


Figure-4



DISCUSSION

Well known metabolic disease, Diabetes affecting people worldwide with uninterruptedly rising trend. As diabetic population is rising, more patients are developing its macro vascular and microvascular complications. According to latest IDF survey, 33 million people in Pakistan are living with diabetes making it third largest diabetic population in world while 11 million people have impaired glucose tolerance test and approximately 8.9 million are undiagnosed diabetic patients.[8] These figures are expected to be doubled by 2025 as per WHO. It is assumed that every fourth person in Pakistan has diabetes so we have a large number of diabetic population. Socioeconomically we are a poor country thus a study about culture isolated in diabetic foot ulcer is worthy to be conducted and possess great significance. Diabetic foot ulceration results due to badly controlled diabetes and poorly managed foot care.[9] It is important to identify microorganisms causing infection in DFU in order to prevent amputation.[10] Diabetic foot infections (DFI) continue to be a widespread issue affecting people of all ages and races. Management of DFI based on many aspects including awareness, etiology, presence of comorbid or risk factors, microbiologic profile, mode of treatment and means to attain prevention against this distressing problem. Studies mostly concentrate on treatment and susceptibility of antibiotic of DFI, ignoring the root cause microorganism responsible for causing infection Identification of microorganism is main objective of this study that will help select appropriate treatment and will ultimately supposed to play a role in curbing the rise in antibiotic resistance.

In our study more 84.3% patients of DFU were older than 46 years (Table-I: age group) while 87.1% of DFI were older than 46 years, this is in concordance with another study in which 93% were older than 40 years[11], may be due to fact that this is the age group mostly affected by diabetes and its long term complications. This is also the age group most commonly affected by obesity and immobility.

In our study 69.6% patients were male and 30.4% were female. Many other studies have shown the predominance of male in DFIs.[12] This difference in prevalence of DFU can be due to increased prevalence of diabetes among male.[13] DFI indicated a slightly higher prevalence in men as compared to women.[14] Additionally, Men also show a higher prevalence of comorbidities that can affect the development of ulcer.[15] A study conducted in Belgium also showed male sex as a risk factor for DFU and DFI as sex influences foot care behavior and personal hygiene. On the other hand, Females manage diabetes carefully. They are generally more aware of complications and are more compliant to care advised to prevent DFU.[16]

In our study there were 102 samples analyzed out of which 75 showed microbial growth, frequency of infected diabetic foot is 73.5%. This frequency is slightly higher than frequency reported in literature which is around 50-60%.[17] Higher infection rate in DFU in our part is most probably due to unhygienic practices, poor self-care, use of alternative medicine, lack of awareness about care of DFU and reluctance to visit professionals, so ulcers get complicated by infections and becomes a major reason for increasing hospital stay, thus increasing treatment cost burden on patient and chances of worse outcomes including amputation.

Among 75 cultures which were positive for microbial growth 29 (38.7 %) cultures were polymicrobial and 46 (61.3%) foot ulcers were monomicrobial. Researches and available literature exhibits predominance of polymicrobial infection in diabetic foot ulcer.[18] In our study most of the DFIs were monomicrobial. Our results are similar to study conducted in north Indian in which 56.6% patients had monomicrobial infection and polymicrobial etiology was observed in 33% while 13.3% showed no growth in their culture report.[19] This study was conducted on a smaller sample size. Monomicrobial infections also predominate in settings where antibiotic is started before sampling for culture and this might be the reason for increased frequency of single microorganism isolation in our study, as most patients visit our hospital after visiting many General practitioners and after taking medicines of homoeopaths and Hakeem which for unknown reasons may mask the isolation of organism. Dhanasekaran et al.'s clinical investigation found that 84% of diabetic foot ulcers are typically monomicrobial.[20] Some studies have reported increased monomicrobial cultures when patients were treated with antibiotics before sampling or improper sampling attributes.[21]

In our study there was a predominance of gram negative isolates 53 (70.7%) while gram positive microbes were isolated from 14 (18.7%) wounds. Mixed gram positive and negative infection was present in 8 (10.7%) cultures which is more than the percentage reported by south Indian research which reported 65% gram negative and 35% gram positive pathogens.[22] In our study, most frequent organism isolated was pseudomonas auraginosa 31 (41.3%) followed by Klebsiella and proteus. Many studies have documented the high prevalence of gram negative isolates from diabetic foot ulcer. A study reported 17 distinct bacterial species identified, with a predominance of Gram-negative bacilli (GNB) in 78.56% of the samples mostly Pseudomonas aeruginosa and Escherichia coli.[23] Another study which was conducted on a large scale with 885 patients included in it, got 1356 pathogen isolates that showed gram-negative organisms predominating, impacting 71.3% of DFIs, while gram-positive bacteria were only detected in 28.7% of DFIs.[24] Previous research from Malaysia, India, and Turkey that found that gram-negative bacteria predominate in DFI supports this conclusion.[25] Conversely, research from the United States and Europe revealed a higher number of DFIs brought on by gram-positive bacteria.[26,27] This difference in microbial pathogen type can be explained on the basis of different geographical location, environmental factors such as hygiene and use of perianal wash. In places where hand washing is common, improper hand washing practices can lead to the contamination of hands with gram-negative bacteria-rich fecal flora. An article by Ramakant et al. supports this opinion.[28] Pseudomonas auraginosa was the most frequent organism isolated followed by Klebsiella and proteus mirabilis. The result is not surprising and predominance of pseudomonas in DFU is supported by many international and local studies. Abdulrazak reported 17.5% among all isolates while India and Pakistan also reported its high rates accounting for 27.05% and 20.1% respectively.[29]

CONCLUSION

Diabetic foot ulcers predominantly get infected by Gram negative bacteria as in other underdeveloped countries.

Although gram positive and mixed infection also contributed to infect DFU in smaller number, most of the infected DFU were monomicrobial and most frequent organism was pseudomonas. Our studies also shows significant male predominance. Proper hygienic practices must be encouraged. Appropriate educational programs targeting awareness for diabetes and related complications must be developed to prevent DFU in this cohort of population.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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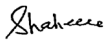
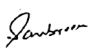
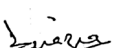
AUTHORSHIP AND CONTRIBUTION DECLARATION

No.	Author(s) Full Name	Contribution to the paper	Author(s) Signature
1	Syeda Hina Abbas	Conception and design, Statistical expertise, Final approval and guarantor of the article.	
2	Syeda Shaheera Zaidi	Conception and design, Statistical expertise.	
3	Nabiya Sandeelo	Collection and assembly of data Analysis and interpretaion of the data.	
4	Sambreen Zameer	Drafting of the article, Critical revision of the article for important intellectual content.	
5	Shazia Nazar	Drafting of the article, Statistical expertise.	
6	Syed Hussain Azhar Rizvi	Collection and assembly of data, Analysis and interpretation of the data.