Isolation and Antibiotics Resistance Pattern of Micro-Organism Recovered From Diabetic Foot Patients

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Abstract:
Background: Diabetic foot infections (DFIs) are a major public health issue and identification of the microorganisms causing such polymicrobial infections is useful to find out appropriate antibiotic therapy.
Aim: To evaluate the bacteriological profile of patients with diabetic foot ulcers and their antibiotic susceptibility pattern
Methods: A cross-sectional study was carried out in the department of microbiology. A total of 120 patients diagnosed with diabetic foot ulcer (DFU) were enrolled. The pus and tissue samples were collected accordance to proper aseptic precautions. Culture, identification of organism and their antibiotic susceptibility pattern was done by Clinical and Laboratory Standards Institute (CLSI) guidelines.
Results: This study of 120 diabetic foot ulcer patients revealed a male predominance. Most patients were in 46-60 years age group. Among gram-positive bacteria staphylococcus aureus whereas among gram negative bacteria pseudomonas and Klebsiella pneumoniae was common. Methicillin resistance Staphylococcus aureus (MRSA) was 87.2% cases. Gram positive cocci were least resistance to vancomycin and Linezolid. E.coli and Klebsiella were most resistance to Ampicillin whereas Pseudomonas was highly resistance to Cefipime and Ceftriaxone whereas least resistance to imipenem and meropenem.
Conclusions: The knowledge on the antibiotic sensitivity pattern of isolates help in planning treatment with the appropriate antibiotic regimen. This, in turn, helps to prevent the emergence of drug-resistant organisms and minimizing healthcare costs.
Keywords: Diabetic foot ulcer (DFU), antibiotic resistance, gram positive bacteria, gram negative bacteria.

Introduction

Diabetic foot is one of the serious complications associated with uncontrolled type 2 diabetes mellitus and affects quality of life in all ages and races patients [1]. The World Health Organization (WHO) reported increasing incidence of diabetes all around the world, especially in developing countries [2].

The vital components involved in treating diabetic foot infections are blood sugar control, treating co-morbidities, broad-spectrum antibiotic therapy, surgical treatment, proper dressing and wound care, personal hygiene, and prevention of recurrence [3-4]. Diabetic foot infections are often polymicrobial. Escherichia coli, Proteus spp., Pseudomonas spp., Staphylococcus aureus and Enterococcus spp. are the most frequent pathogens contributing to progressive and widespread tissue destruction [5-

6]. Patients with poorly treated DFU may develop diabetic foot osteomyelitis leading to gangrene and amputation. Diabetic foot infections (DFI) are predominantly polymicrobial and multidrug resistant (MDR) with the ability to form biofilm [7]. Foot ulcer commonly affects people with type II DM. It can lead to infection and amputation of lower extremities. The risk of developing an ulcer increases with peripheral vascular disease, neuropathy, diabetes duration ≥ 10 years, insulin use, retinopathy, nephropathy, age 45 years, cerebral vascular disease, and poor glycemic control.

Increasing cumulative glycemic burden, coronary artery disease, male gender, smoking, and hypertension are all present in these patients [8-9]. In recent decade, high rates of MDR bacteria,

MRSA, and ESBL positive strains have been observed in many hospitalized diabetic foot patients (DFP) [10]. Adequate knowledge about the microbes that cause infection is very important and helps in determining appropriate antibiotic therapy and proper management of these infections. Hence, the study was carried out to evaluate the microbiological characteristics of diabetic foot ulcers and their susceptibility pattern to various antimicrobials

Aims & objectives:

This study investigates the microbial causes of diabetic foot infections and their antibiotic susceptibility pattern in a tertiary care hospital

Materials and Methods

This cross sectional observational study was conducted at Department of Microbiology, in a tertiary care teaching hospital, Chhattisgarh, India. Eighty patients with DFU attending the diabetic foot clinic during the study period were recruited for this study.

All study participants provided written informed consent prior to their involvement. As per the criteria set by the International Working Group on the Diabetic Foot (IWGDF), a DFU was defined as a full-thickness wound that extends through the dermis and is situated below the ankle in individuals with diabetes [11].

Inclusion criteria:

- Patients aged 18 years or more with both gender
- DFU Patients provided their consent to participate

Exclusion criteria

- Patients <18 years of age
- Patients with non-diabetic foot ulcer and repeat isolates from same patient
- Patients who not provided their consent for the study

Socio-demographic information was collected and relevant investigations were done from all the patients. Fasting blood glucose levels equal to or greater than 126 mg/dL and glycosylated hemoglobin (HbA1c) levels equal to or greater than 7% were considered abnormal.

Two specimens (pus, wound exudates) for microbiological studies were obtained from the infected sites following a specific protocol. One swab was used for Gram staining and second used for culture. The samples were inoculated onto Blood agar & MacConkey agar and incubated at 37 degrees Celsius under aerobic conditions for 24-48 hours.

Subsequently, the obtained colonies were identified, and antibiotic sensitivity was determined using the Kirby-Bauer's disc diffusion technique, as outlined in the Clinical Laboratory Standard Institute guideline [12].

Statistical analysis:

The statistical analysis of the data involved both descriptive and inferential statistics. The Chi-square test was utilized for the inferential analysis. The software programs used for the analysis were SPSS (Statistical Product and Service Solutions) version 18.0 and Graph Pad Prism version 5.0.

Statistical analysis:

All data was compiled and presented in tabular and/or graphical form, percentages were calculated wherever relevant. Data was analyst using SSPS version 22. Fischer's exact test was used. A significance level of $P < 0.05$ was considered to determine statistical significance in the results

Results

A total of 120 diabetic foot patients were enrolled in this study. The majority of the patients (34.2%) were 46-60 years age group, predominantly male (68.3%). The preponderance of cases involved Type II Diabetes (95.8%), with most of them (45%) duration of diabetes was 5-10 years. Pus specimen was collected in 74.2% of cases and 65% of participants received insulin therapy. Details description was shown in Table 1.

Table 1: Demographics and clinical features of study population

Variable		Number (n=120)	Percentage (%)
Age (years)	18-30	24	20%
	31-45	32	26.6%
	46-60	41	34.2%
	61-75	23	19.2%
Gender	Male	82	68.3%
	Female	38	31.7%
Type of Diabetes	Type I	5	4.2%
	Type II	115	95.8%
Duration of Diabetes	<5 years	25	20.8%
	5-10 years	54	45%
	10-15 years	30	25%

	>15 years	11	9.2%
Specimen Collected	Pus	89	74.2%
	Exudates/tissue	31	25.8%
Drugs	Insulin	78	65%
	Oral Antidiabetic	42	35%

Organism isolated from diabetic foot ulcer patients were polymicrobial in 58% and Monomicrobial in 42% cases.
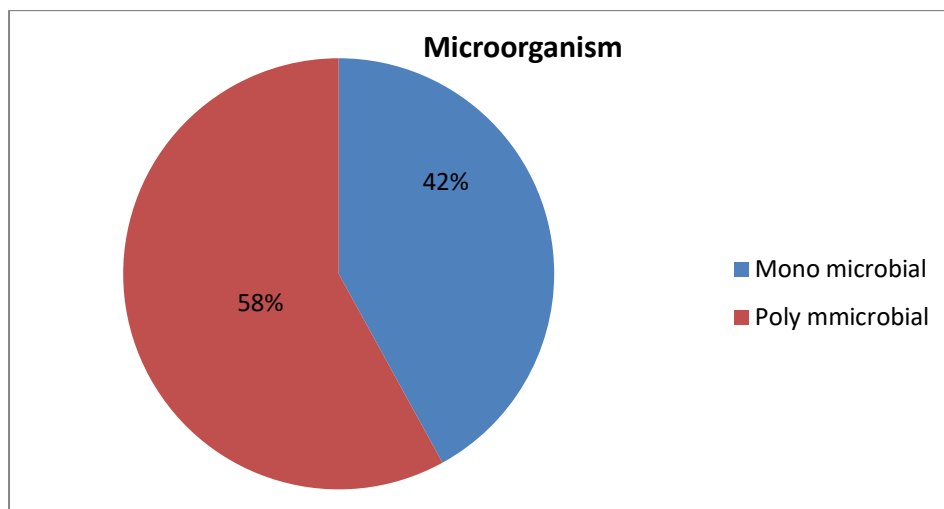

Figure 1: Microorganism isolated from Diabetic foot ulcer

A total of 132 organisms were isolated from 120 diabetic foot patients. Staphylococcus aureus was predominant (29.5%) followed by Coagulase negative staph (CONS) 9.8% among gram positive bacteria, whereas among gram negative bacilli Pseudomonas aeruginosa (15.9%) was common followed by Klebsiella pneumoniae (13.6%) cases. Candida species was isolated in 1.5% cases. Details were shown in table 2.

Table 2: Profile of Microorganism isolated from Diabetic foot ulcer

Type of Isolate	Frequency (n=132)	Percentage
Gram Negative organism		
Klebsiella pneumoniae	18	13.6%
Pseudomonas aeruginosa	21	15.9%
Escherichia coli	14	10.6%
Proteus species	5	3.7%
Acinetobacter	7	5.3%
Citrobacter species	3	2.2%
Gram positive organism		
Staphylococcus aureus	39	29.5%
CONS	13	9.8%
Streptococcus species	7	5.3%
Enterococcus species	2	1.5%
Fungal organism		
Candida species	2	1.5%
Aspergillus species	1	0.7%

Antibiotic resistance pattern of gram positive cocci isolated from DFU patients indicate that these organisms exhibited the highest resistance to Cefoxitin and Ampicillin, whereas least resistance to Vancomycin and Linezolid. Methicillin resistance staph aureus (MRSA) was observed in 87.2% of cases. Detail of antibiotics resistance pattern shown in table 3.

Table 3: Antimicrobial resistance pattern of Gram positive cocci

Antimicrobial agent	Staphylococcus aureus (n=39)	CONS (n=13)	Streptococcus species (n=7)
Ampicillin	35 (89.7%)	10 (76.9%)	6 (85.7%)

Cefoxitin	34 (87.2%)	10 (76.9%)	6 (85.7%)
Clindamycin	25 (64.1%)	7 (53.8%)	3 (42.8%)
Levofloxacin	17 (43.5%)	7 (53.8%)	4 (57.1%)
Tetracycline	19 (48.7%)	6 (46.1%)	3 (42.8%)
Gentamicin	31 (79.4%)	9 (69.2%)	5 (71.4%)
Vancomycin	3 (7.6%)	2 (15.4%)	1 (14.3%)
Erythromycin	20 (51.2%)	5 (38.5%)	3 (42.8%)
Linezolid	2 (5.1%)	2 (7.7%)	1 (14.3%)

Antibiotic resistance pattern of gram negative bacilli isolated from DFU patients exhibited the maximum resistance to Ceftriaxone and Cefipime whereas minimum resistance to Imipenem and meropenem [Table 4].

Table 3: Antimicrobial resistance pattern of Gram negative bacilli

Antimicrobial agent	Pseudomonas aeruginosa (n=21)	Klebsiella pneumoniae (n=18)	Escherichia coli (n=14)
Aztreonam	9 (42.80%)	7 (38.8%)	6 (42.8%)
Cefipime	18 (85.7%)	13 (72.2%)	10 (71.4%)
Ceftriaxone	-	16 (88.8%)	11 (78.5%)
Ceftazidime	5 (23.8%)	5 (27.7%)	3 (21.4%)
Imipenem	7 (33.3%)	8 (44.4%)	5 (35.7%)
Ciprofloxacin	12 (57.2%)	11 (61.2%)	7 (50%)
Meropenem	4 (19.1%)	3 (16.6%)	2 (14.2%)
Piperacillin/Tazobactum	6 (28.5%)	6 (33.3%)	6 (42.8%)

Discussion

Diabetic foot ulcer is an important complication of DM. Untreated diabetic foot ulcers will become infected leading to various other consequences such as gangrene or amputation of the limb. Surgical intervention and treatment with antibiotic regimen are the options used for the management of DFUs [13]. Clinicians face the added challenge of treating diabetic patients with nephropathy, a condition that affects approximately one-third of individuals with diabetes. Furthermore, the increasing incidence of multidrug-resistant infections in diabetic foot ulcers further compounds the difficulties faced by healthcare professionals in providing effective treatment [14].

The majority of diabetic foot ulcer patients in the current study (68.3%) were male; these results are in line with those of previous studies conducted by Murshed M, et al [15] and Shah P, et al [16]. This could be explained by the greater active participation of males in outdoor sports, which exposes them to accidents and ulcer formation. This study observed that most of the diabetic foot ulcer patients was 46-60 years age group, our finding corroborate with the Appapalam et al [17] and Patil SV, et al [18]. The foot infections of diabetic individuals in the late 50s might be a result of neuropathy, vasculopathy, and reduced immune response.

In our pus samples were collected from the majority of the DFU patients, similar results were obtained by R Prakash et al [19]. Present study found that majority of samples collected from the DFU patients were of grade 2 polymicrobial infections, in agreement with the Mama M, et al [20] and Karmaker M, et al [21]. The recent study shows that a variety of organisms can infect diabetes individuals, but Gram negative bacteria are the most persistent and hazardous pathogens that cause systemic symptoms. Gram negative bacteria made up a higher percentage than Gram positive bacteria, our results comparable with the Neha G, et al [22] and Akhi MT, et al [23]. Current study reported that Pseudomonas aeruginosa was the most frequent followed by Klebsiella pneumonia and E.coli among gram negative bacteria isolated from DFU patients, consistent finding observed by Manikandan C, et al [24] and Muamar M, et al [25].

Staphylococcus aureus was the predominant pathogen among gram positive bacteria in this study, concordance with the Citron et al [26] and Abdulrazak et al [27]. Current study observed 87.2% cases were resistant to Cefoxitin (MRSA). The results of this investigation corroborated those of Dwedar et al [28]. In the present study, Imipenem and Meropenem showed very good susceptibility against Pseudomonas and Klebsiella species, our results correlates with the other studies like Shareef, et al [29] and M Anvarinejad, et al [30]. The present investigation confirmed that DFU infection is caused by both Gram positive and Gram negative bacteria. Due to their antimicrobial resistance profile, these bacteria can pose difficulties for patient management and increase complications like osteomyelitis and potentially necessitate amputation of the limb.

Conclusion

This study showed the staphylococcus and predominance were with the common organism isolated from the DFU mostly polymicrobial in nature. Knowledge about the antibiotic susceptibility pattern of the isolates is also essential for proper management of diabetic foot infections. When selecting an antibiotic for early treatment, the majority of practitioners will prescribe an antibiotic based on their expertise and observations. Prior to treatment, a proper understanding of antibiotic resistance will aid in the effective management of the illness.

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