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Microbial profile and antibiogram of isolates from diabetic foot ulcer in Tertiary Health Care Hospital of Central India

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Abstract

To estimate the microbial profile and antibiogram of isolates from diabetic foot ulcer in tertiary care hospital of central India. This is a cross-sectional observational study conducted in patients presenting with diabetic foot ulcer in OPDs and admitted in tertiary care hospital of Chhattisgarh. Foot ulcer grading was done using Wagner's classification. Samples processing, isolation and identification of positive isolates was done using standard microbiological procedures. Antimicrobial susceptibility was evaluated using CLSI guidelines. Total 30 patients of diabetic foot ulcer were included. Most common age group affected was 31-60 years (66.7%) followed by 61-90 years (26.7%). Male to female ratio was 2:1. Mean duration of diabetes was 3.36 years with minimum duration 10 days and maximum duration 20 years in study patients. According to Wagner's grading, most common grade of ulcer was grade 1 (33.3%) followed by grade 2 (30%) and 3(30%). Peripheral neuropathy was associated in all cases and 43.3% showed association with hypertension. Most common site of diabetic ulcer was right foot 21 (70%) and dorsum of foot 17 (56.7%). Most common organism isolate was *Pseudomonas aeruginosa* (30%), followed by *E. coli* (25%) and *Klebsiella pneumoniae* (25%). Among Gram positive most common organism isolate was *Staphylococcus aureus* (15%). Imipenem and Meropenem were found to be effective drugs for Gram-negative organisms. For Gram-positive coverage Gentamycin and cephalosporins was found to be effective. Early diagnosis, prompt patient care and implementation of strict antimicrobial stewardship practices are crucial for treatment of diabetic foot ulcer.

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Introduction

Diabetes mellitus is one of the leading and chronic metabolic disorders which affect various mass of people, not only in India, but also across the world (American Diabetes Association, 2011). According to Wild *et al.,* India has reported about 50.8 million cases of diabetes in across both sexes and it is expected to rise up to 87 million by 2030 (Wild *et al.*, 2004). Diabetes mellitus type 2 is most common form of diabetes in developing countries like- India which appears to be the diabetic capital of the world (Hu FB, 2011). The prevalence of diabetes in India is to be 7.3% and pre diabetic population is to be around 10.3%. In India the rural population shows prevalence of diabetes about 5.2%, while in urban population it is about 11.2% (ICMR-INDIAB, 2017).

Diabetic foot disease represents a real challenge to national health systems and healthcare providers in general (Mariam *et al.*, 2017). The lifetime risk of a person with diabetes having a foot ulcer has been reported to be as high as 25%, with foot ulcers being the most frequent reason for hospitalization of patients with diabetes (about 30%) (Sinharay *et al.*, 2012). Moreover, treating diabetic foot ulcers is costly, accounting for 20% of total healthcare costs for diabetes, which is more compared to the cost for any other diabetic complication (Sinharay *et al.*, 2012). In India, the numbers of diabetic foot patients are increasing in both urban and rural settings, with 85% of amputations preceded by foot ulcers. Almost 75% of these amputations are performed on neuropathic feet with secondary infection, which is potentially preventable (Tripathy *et al.*, 2017).

This study will give picture of microbiological profile and antimicrobial susceptibility pattern of isolates from cases suffering from diabetic foot ulcer along with associated epidemiology of disease in existing scenario in our region, which will not only help to determine the appropriate empirical therapy but also is crucial for antimicrobial stewardship.

To estimate the microbial profile and antibiogram of isolates from diabetic foot ulcer in tertiary care hospital of central India.

Material & methods

Study design

Cross-sectional observational study

Study location

Tertiary care teaching hospital of Chhattisgarh

Study duration

3 months from 6 July 2018 to 6 September 2018

Inclusion criteria

Diabetic foot ulcer cases taken from both outpatient department and patients admitted in department of Surgery and Medicine of tertiary care teaching hospital.

Exclusion criteria

Those with critical illness and having sepsis or gangrene of feet were excluded.

Sample collection

A total of 30 samples (including pus swabs, debrided ulcer material or aspirate of pus from base of ulcer) from different cases of diabetic foot ulcer collected during study period, which showed positive growth on culture were examined in Microbiology Department of Government Medical College after taking proper informed consent from each patient.

Detailed history of the patient was collected for epidemiological purposes. Foot ulcer grading was done using Wagner's ulcer classification system.

Ulcer grading	Description
Grade 0	No ulcer but high-risk foot
Grade 1	Superficial ulcer
Grade 2	Deep ulcer, no bony involvement or abscess
Grade 3	Abscess with bony involvement (as shown by X-ray)
Grade 4	Localized gangrene e.g. toe, heel etc
Grade 5	Extensive gangrene involving the whole foot

Note: Grade 1–3 ulcers are termed *non-gangrenous ulcers* and Grade 4 and 5 ulcers are termed *gangrenous ulcers*

Sample processing

Isolation and identification of microorganism was done following standard microbiological procedures. All samples collected were cultured onto blood agar and MacConkey agar and incubated overnight at 37C. Single pure isolated colonies grown were examined under microscope after Gram staining.

Identification

Colonies of Gram-Positive cocci were further subjected to catalase test, modified oxidase test, slide coagulase test, tube coagulase test, bile esculin test a long with optochin and bacitracin sensitivity testing. Colonies of Gram- negative isolates were subjected to catalase test, oxidase test, indole test, MR-VP test, citrate test, triple sugar iron test, and oxidation - fermentation test along with sugar utilisation and amino acid utilization tests.

Antibiotic susceptibility test (AST)

AST of the isolates was done by Kirby- Bauer disc diffusion method as per the CLSI guidelines 2018. The antimicrobial discs which were used were Gentamicin (10µg), Tobramycin (10µg) Amikacin (30µg), Levofloxacin (5µg), Ciprofloxacin (5µg), Chloramphenicol (30µg), Ceftazidime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Cotrimoxazole (1.25/23.75 µg), Piperacillin/tazobactam (100/10µg), Imipenem (10µg), Meropenem (10µg) - for the Gram-negative bacilli. Cefoxitin (30µg), Chloramphenicol (30µg), Clindamycin (2µg), Linezolid (30µg) and Gentamycin (10µg) were used to study the susceptibility patterns of the Gram-positive cocci. Detection of resistance mechanism for MRSA, Vancomycin resistance, ESBL, Amp-C-β Lactamase and carbapenemase production was done using methods dictated in CLSI guidelines 2018.

Statistical analysis

Quantitative data was expressed as mean and SD. Qualitative variables were expressed as percentage. A descriptive analysis of the data was done.

Picture 1. Pictures showing diabetic foot ulcer presentation from some of cases presenting in government medical college.

Results

Demographic Profile

Total 30 patients of diabetic foot ulcer were included in our study. Amongst all patients, most common age group was 31-60 years (66.7%) followed by 61-90 years (26.7%) (Fig. 1). Male (67%) patients were more than female patients (33%) (Fig. 2). Male to female ratio was 2:1.

Mean duration of diabetes was 3.36 years with minimum duration 10 days and maximum duration 20 years in study patients (Fig. 3). According to Wagner's grading, most common grade of ulcer was grade 1 (33.3%) followed by grade 2 (30%) and 3(30%) (Fig. 4). All the study patients had complication of diabetes as neuropathy (100%) and 43.3% showed associated hypertension (Fig. 5). Most of study patients had non necrotic ulcer 22 (Fig. 6). Most common site of diabetic ulcer was right foot 21 (70%) and dorsum of foot 17 (56.7%) (Fig. 7).

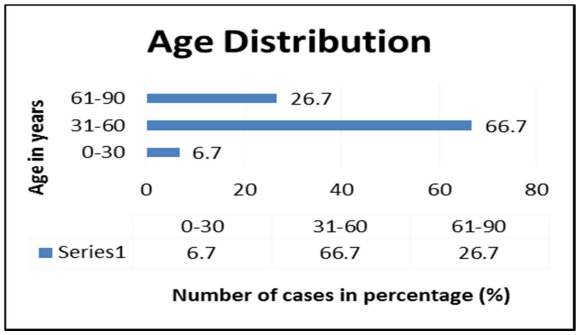

Fig. 1. Graph showing age distribution of patients in our study.

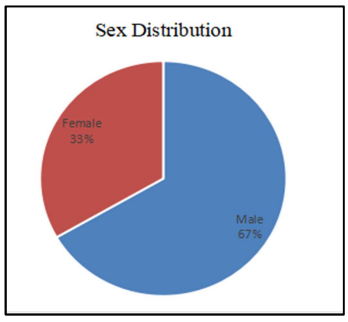

Fig. 2. Graph showing sex distribution of patients in our study.

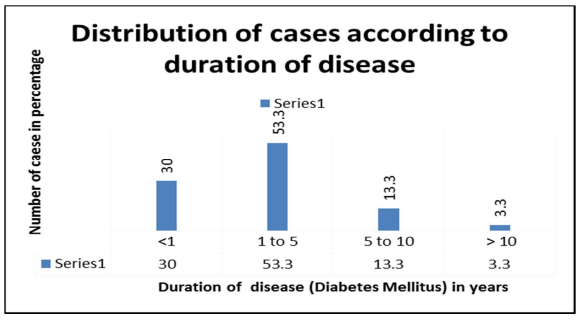

Fig. 3. Distribution of cases according to duration of disease.

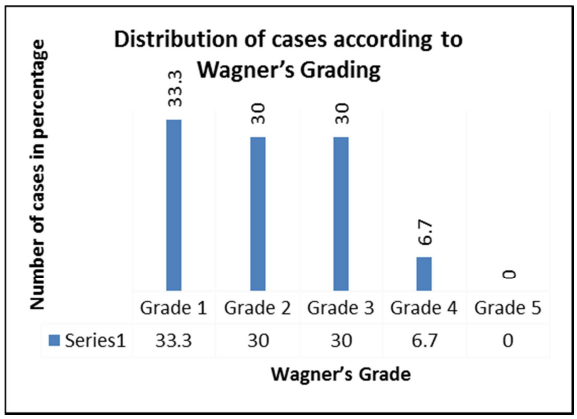

Fig. 4. Distribution of cases according to Wagner's grading.

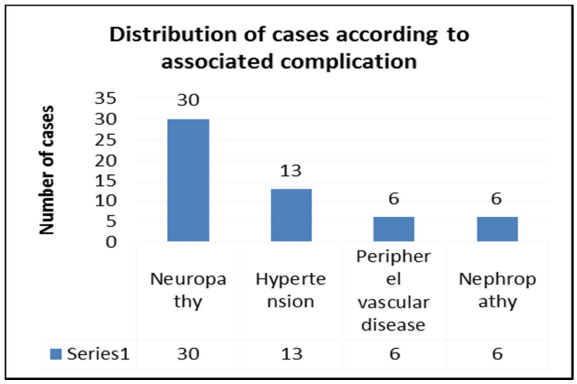

Fig. 5. Distribution of cases according to associated complication.

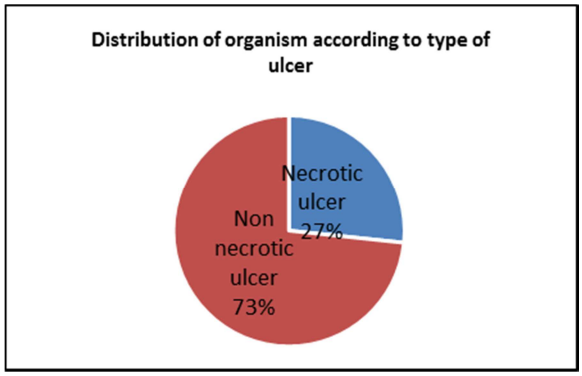

Fig. 6. Distribution of cases according to type of ulcer.

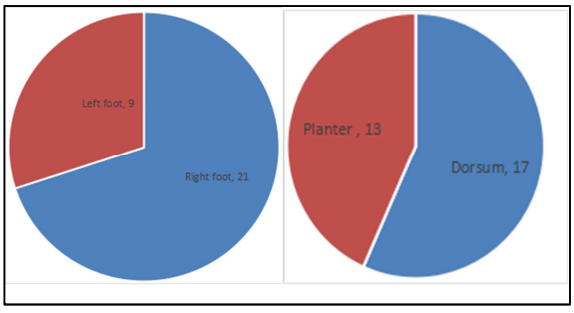

Fig. 7. Distribution of cases according to site of ulcer.

Microbiological Profile

Table 1. Spectrum of organism.

Gram Positive Organism	No. of patients
Staphylococcus aureus	3 (15%)
Escherichia coli	5 (25%)
Pseudomonas aeruginosa	6 (30%)
Klebsiella pneumonia	5 (25%)
No growth	11

Antimicrobial Susceptibility pattern

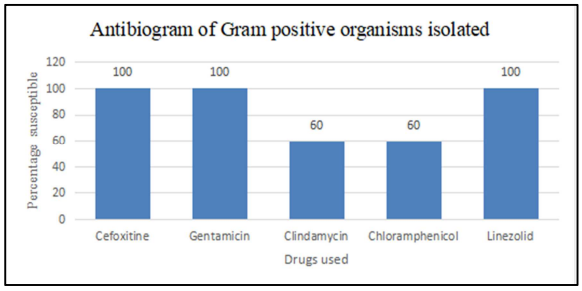

Fig. 8. Antibiogram of Gram-positive isolates (% susceptible).

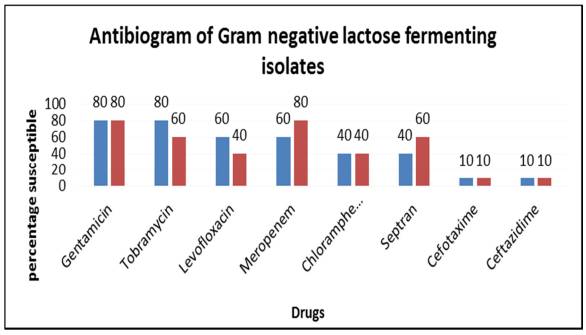

Fig. 9. Antibiogram of Gram-negative lactose fermenters (in% susceptible).

Gram negative bacteria was the most common isolate observed. Among gram negative organism, most common organism isolate was *Pseudomonas aeruginosa* (30%), followed by *E, coli* (25%)and *Klebsiella pneumoniae* (25%) (Table 1). Among Gram positive most common organism isolate was *Staphylococcus aureus* (15%) (Table 1).

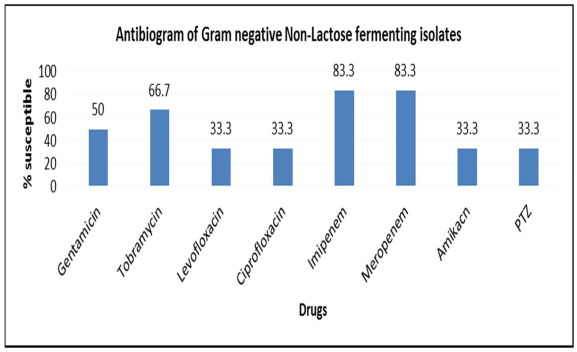

Fig. 10. Antibiogram of Gram negative non lactose fermenters (in% susceptibility).

In our study all staphylococci were resistant to penicillin, while none showed resistance to cefoxitin, gentamicin and linezolid (Table 2). *E. coli* showed 80% sensitivity to gentamicin and tobramycin followed by levofloxacin (60%) and meropenem (60%). *Klebsiella pneumoniae* showed 80% sensitivity to gentamicin and meropenem and 60% sensitivity to tobramycin and septran (Table 3). *Pseudomonas aeruginosa* showed a high degree of resistance to most of the antibiotics but was sensitive to imipenem (83.3%), meropenem (83.3%), tobramycin (66.67%) (Table 4).

Table 2. Antimicrobial Susceptibility of Gram-positive isolates

Organism	Cefoxitin	Gentamicin	Clindamycin	Chloramphenicol	Linezolid
S. aureus	3 (100%)	3 (100%)	2 (60%)	2 (60%)	3 (100%)

Table 3. Antimicrobial Susceptibility of Gram-negative lactose fermenter isolates

Organism	Gentamicin	Tobramycin	Levofloxacin	Meropenem	chloramphenicol	Cotrimo-xazole	cefotaxime	ceftazidime
E. coli	4(80%)	4(80%)	3(60%)	3(60%)	2(40%)	2(40%)	1(10%)	1(10%)
K.pneumoniae	4(80%)	3(60%)	2(40%)	4(80%)	2(40%)	3(60%)	1(10%)	1(10%)

Table 4. Antimicrobial Susceptibility of Gram-negative non lactose fermenter isolates

Organism	Gentamicin	Tobramycin	Levofloxacin	Ciprofloxacin	Imipenem	Meropenem	amikacin	Piptaz
P.aeruginosa	3(50%)	4(66.67%)	2(33.3%)	2(33.3%)	5(83.3%)	5(83.3%)	2(33.3%)	2(33.3%)

Antimicrobial resistance

All Gram positive isolates were found susceptible to methicillin and other beta lactam drugs while out of the Gram negative lactose fermenters , 2 (20%) of isolates were found to be extended spectrum beta lactamase producers.

Discussion

Diabetes mellitus is one of the major public health problems whose prevalence is rapidly rising all over the globe at an alarming rate. Nowhere is the diabetic epidemic more pronounced than in India, as the WHO reports show that 69.2 million people had diabetes in the year 2015 and it is expected to rise up to 87 million by 2030. Diabetic foot ulcer is the most common complication of diabetes mellitus. It may develop as a result of neuropathy, ischemia or both and when infection complicates a foot ulcer, the combination can become life threatening (WHO, 2023).

Diabetic foot ulcers are one of the most distressing complications of diabetes affecting around 15% of people with diabetes. The annual incidence of diabetic foot ulcers is around 3% and the reported incidence in U.S and U.K studies ranges as high as 10%. It has been reported that 85% of the lower limb amputations in diabetic patients are preceded by foot ulceration (Paras and Hameed, 2020).

The higher prevalence of foot ulcers in the late 50's might be due to the occurrence of neuropathy, vasculopathy and altered immune responses in diabetic individuals and they are more evident in the later age groups as the disease progress. Similar result was found in study conducted by Ravoori *et al.* (2023). Male preponderance in the present study could be explained on the basis that the males spend more time working outdoors, exposing their foot to more traumas. This observation was comparable with the studies of (Ramani *et al.*, 1991).

The present study showed that the mean duration of diabetes was 3.36 years which is very less (Fig. 3). This could be due to lack of health education among population leading to delayed diagnosis of diabetes.

Diabetic patients often undergo chronic long lasting and non-healing ulcers due to various underlying factors such as neuropathy, high plantar pressures and peripheral arterial diseases, such factors make the patients prone to certain bacterial infections that lead to delayed wound healing process. In our study we found that peripheral neuropathy (100%) was commonly associated risk factor for the development of foot ulcers followed by hypertension (Fig. 5). This study result matches with (Viswanathan, 2010).

Pseudomonas aeruginosa and other Gram-negative lactose fermenters were found to be most common causative agents. Imipenem and Meropenem were found to be effective drugs for Gram-negative organisms. For Gram-positive coverage Gentamycin and cephalosporins was found to be effective. Although no MRSA was isolated but many of the Gram-negative isolates were found to be multi drug resistance which calls for implementation of strict antimicrobial stewardship practices.

Early diagnosis and prompt patient care are crucial for treatment of diabetic foot ulcer. Thus, there is a need of larger multicenter study for evaluation of microbiological profile and antimicrobial susceptibility pattern in diabetic foot ulcer patients in order to improve treatment outcome and reduce morbidity by reducing need for amputations.

Conclusion

There is a high occurrence of foot ulcers within the population of people with diabetes. Foot ulcerations may lead to infections, lower extremity amputations and are major causes of disability to patients, often resulting in significant morbidity, extensive periods of hospitalization,

and mortality. In order to diminish the detrimental consequences associated with diabetic foot ulcers, a high standard of care must be provided and appreciation of the causative organisms in diabetic foot and their antibiotic sensitivity is essential for institution of appropriate antibiotic therapy. There is great need for implementation of strict antimicrobial stewardship practices in order to curb the menace of antimicrobial resistance.

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