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Bacteriological profile and antimicrobial sensitivity pattern of isolates from diabetic foot of patients attending a teaching hospital in Northern India

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ABSTRACT

Background: Diabetic foot ulcer is a major cause for diabetes related morbidity and hospitalization. Up to one-third of people with diabetes develop diabetic foot ulceration (DFU) during their lifetime and over 50% of these ulcerations become infected. Diabetic foot infections (DFIs) are associated with major morbidity, increasing mortality, high costs, increased risk of lower extremity amputation (LEA), and reduced quality of life. **Aims and Objective:** The current study was conducted to determine the microbiological profile and antibiotic susceptibility pattern of organisms in diabetic foot ulcers patients at a tertiary care center in Srinagar province. **Materials and Methods:** This was a Cross-sectional, observational study conducted in diabetic patients with diabetic foot infection, randomly selected from outpatient departments (OPDs) and wards of Surgery and Medicine department, with Wagner grade 1-5 ulcers and irrespective of anti-diabetic treatment and diabetic foot injury treatment. Samples were processed and bacterial isolates were identified by standard microbiological procedures. **Results:** After following inclusion and exclusion criteria, 120 patients were considered for this study. In present study most common age group was 51-60 years age group (44%) followed by 41-50 years (32%). 66% of participants were males. 55% patients had diabetes for more than 10 years. 43% patients had ulcer size less than 5 cm^2. The most common category as per Wagner's classification was Grade 1, which comprised of 48% of study participants, followed by Grade 0 (28%) and Grade 2 (18%). Grade 3 and above comprised 7% of cases. Of the 120 study participants, 103 (86%) showed growth on culture. Among these 62 (60%) showed mono-microbial growth with 41 cases showing mixed growth. The most commonly isolated bacteria were Methicillin Resistant *Staphylococcus aureus* (MRSA) (23%), Coagulase Negative *Staphylococci* (CoNS) (18%), *pseudomonas aeruginosa* (18%), Methicillin Sensitive-*Staphylococci Aureus* (9%), *Klebsiella Pneumoniae* (9%), and *Escherichia Coli* (8%). Linezolid, vancomycin, clindamycin, gentamicin were most effective antimicrobial agents against gram positive bacteria. Iimipenem, piperacillin tazobactam, cefoperazone sulbactam & gentamicin were most effective antimicrobial agents against gram negative bacteria. **Conclusion:** Early microbiological evaluation for bacteriological profile, the nature of the infection either monomicrobial or polymicrobial and antibiotic sensitivity testing can improve treatment outcome, reduces complications, morbidity as well as multidrug resistance.

Key words: Diabetic foot; Diabetic foot ulcer; Diabetic foot infection; Wagner's grading

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INTRODUCTION

Diabetes mellitus is most common endocrine disorder with common affection towards kidney, nervous system and skin.[1] Diabetes mellitus is a worldwide problem and its prevalence is increasing due to sedentary lifestyle, junk foods, obesity, early diagnosis, etc.

Metabolic abnormalities of insulin and elevated blood glucose level leads to multiple vascular, neurological and immunological abnormalities mainly affecting cardiovascular, renal and nervous systems, eyes and the skin. Diabetic foot injuriess arise mainly from skin ulceration associated with loss of protective sensation (peripheral neuropathy), altered foot architecture, and some forms of trauma. Various types of microorganisms colonize and proliferate on the wounds, which serve as a point of entry, causing tissue damage and infection.[2]

Diabetic foot ulcer is a major cause for diabetes related morbidity and hospitalization. It is estimated that approximately 20% of hospital admissions among diabetic patients are due to diabetic foot ulcers & related complications.[3] Up to one-third of people with diabetes develop a diabetic foot ulceration (DFU) during their lifetime and over 50% of these ulcerations become infected.[4] Diabetic foot infections (DFIs) are associated with major morbidity, increasing mortality, high costs, increased risk of lower extremity amputation (LEA), and reduced quality of life.

Most of these infections are polymicrobiol in nature and mixed organisms are frequently encountered. However, the spectrum of microorganisms depends mainly on microbial flora of lower limb, metabolic factors, foot hygiene and the use of antibiotics. Diabetic foot infections may be extremely challenging to cure, due to late diagnosis (due to blunted clinical signs), presence of ischemia, difficult to-treat multidrug-resistant pathogens, and spread of infection to the bones, leading to osteomyelitis.

The present study was conducted to determine the microbiological profile and antibiotic susceptibility pattern of organisms in diabetic foot ulcers patients at a tertiary care center in Srinagar province

MATERIAL AND METHODS

Present study was a cross-sectional, observational study conducted in the Department of Microbiology, Government Medical College and Hospital, Srinagar. Study period was September 2018 to August 2019 (1 year).

Inclusion criteria was all diabetic patients with diabetic foot infection were randomly selected from outpatient departments (OPDs) and wards of Surgery and Medicine department, with Wagner grade 1-5 ulcers and irrespective of anti-diabetic treatment and diabetic foot injury treatment, willing to participate in study.

Exclusion criteria were those patients who were **s**seriously ill, patients with neuropathy other than diabetic neuropathy, patients with acute limb ischemia, patients not willing to participate and inadequately collected sample.

Study was explained to patients and a written informed consent was taken from patients. The clinical details of the patients such as age, sex, types of diabetes, duration of diabetes, size of ulcer and duration of ulcer were recorded. The ulcers were graded according to the Wagner's grade classification.

Samples (pus, debrided ulcer material or aspirate of material from infected wound) from the infected foot lesions were collected aseptically by using sterile cotton swab. Sterile cotton swab sticks were moistened with sterile normal saline before collecting the specimens. The swab sticks were extended deeply into the depth of the lesion avoiding touching of surrounding skin area around the wound. The collected samples were immediately transported to the microbiology department.

Samples were processed & bacterial isolates were identified by standard microbiological procedures (Macroscopic evaluation, Microscopic examination, culture, motility and biochemical test) and antibiotic susceptibility testing was performed through the Kirby Bauer's disc diffusion method.

Results of the culture and antimicrobial sensitivity testing were documented. Collected data was entered in Microsoft excel sheet & analysed. Statistical analysis was done using descriptive statistics.

RESULTS

After applying inclusion & exclusion criteria, total 120 patients was considered for study. In present study most common age group was 51-60 years age group (44%) followed by 41-50 years (32%). Male patients (66%) were more than female (39%). Male to female ratio was 1.9:1. History of trauma (48%), family history of diabetes (26%), hypertension (19%), smoking (18%) & alcohol consumption (16%) were common risk factors in study patients. 55% patients had diabetes for more than 10 years. 43% patients had ulcer size less than 5 cm^2 (Table 1).

The most common category as per Wagner's classification was Grade 1, which comprised of 48% of study participants, followed by Grade 0 (28%) and Grade 2 (18%). Grade 3 and above comprised 7% of cases (Table 2).

Microbiological profile

Of the 120 study participants, 103 (86%) showed growth on culture. Among these 62 (60%) showed mono-microbial growth with 41 cases showing mixed growth. The most commonly isolated bacteria were Methicillin Resistant *Staphylococcus Aureus* (MRSA) (23%), Coagulase Negative *Staphylococci* (CoNS) (18%), *pseudomonas Aeruginosa* (18%), Methicillin Sensitive-*Staphylococci Aureus* (9%), *Klebsiella Pneumoniae* (9%), and *Escherichia Coli* (8%) (Table 3).

In vitro sensitivity of antimicrobial agents against Gram positive bacteria was done. Linezolid, vancomycin, clindamycin, gentamicin were most effective antimicrobial agents against methicillin resistant staphylococcus aureus (MRSA), coagulase negative staphylococci (CoNS) and methicillin sensitive-staphylococci aureus (9%) (Table 4).

In vitro sensitivity of antimicrobial agents against gram negative bacteria imipenem, piperacillin tazobactam, cefoperazone sulbactam & gentamicin were most effective antimicrobial agents against pseudomonas aeruginosa, klebsiella pneumoniae and escherichia coli.

Table 1: Demographic profile and risk factors of diabetic foot patients

Characteristics	No of patients	Percentage
Age Group (in years)		
21-30	1	1%
31-40	3	3%
41-50	38	32%
51-60	53	44%
61-70	17	14%
71-80	8	7%
Mean Age(years)	52.7 ± 11.3	
Sex		
Male	79	66%
Female	41	34%
Risk factors		
History of trauma	58	48%
Family History of diabetes	31	26%
Hypertension	23	19%
Smoking	21	18%
Alcohol consumption	19	16%
Duration of diabetes mellitus		
<5 years	19	16%
5-10 yrs.	35	29%
>10 years	66	55%
Size of ulcer (in cm^2)		
<5	51	43%
5-20	43	36%
>20	26	22%

Table 2: Wagner's classification in diabetic foot patients

Grade	Clinical signs	Number	%
0	Intact skin	33	28
1	Superficial ulcer of skin/ subcutaneous tissue	58	48
2	Ulcer extending to tendon/ bone/ capsule	21	18
3	Deep ulcer with osteomyelitis/ abscess	5	4
4	Gangrene of toes/ forefoot/ localized gangrene	3	3
5	Mid foot/ hind foot gangrene	0	0
	TOTAL	120	

DISCUSSION

Diabetic foot ulcers are not spontaneous ulcers, but results from the interplay of various factors line neuropathy, autonomic neuropathy, and peripheral vascular disease, superimposed with alterations in the plantar pressure, defective footwear and limited joint mobility.

Majority of diabetic foot injuries in India are due to sociocultural practices such as barefoot walking, religious practices like walking on fire, use of improper footwear and lack of knowledge regarding foot care attributes towards increase in the prevalence of diabetic foot.[5] The combination of insensate foot secondary to neuropathy and deformation predispose the diabetic patients to ulcers formation. The ulcers are colonised by the bacteria and when the overgrowth of the pathogens triggers the deleterious inflammation or tissue destruction, it is termed as an infection.[6]

Hyperglycemia, neuropathy, peripheral arterial disease, foot deformity, foot trauma, impaired immunologic response and infections are the major predisposing factors leading to

Table 3: Bacterial isolates

Organism isolated	Number	Percentage
Gram positive organisms (60%)		
Methicillin resistant Staphylococcus aureus	27	23%
Coagulase Negative Staphylococci	21	18%
Methicillin sensitive-Staphylococci aureus	11	9%
Group B Streptococci	8	7%
Enterococcus faecalis	5	4%
Gram negative organisms (49%)		
Pseudomonas aeruginosa	22	18%
Klebsiella pneumoniae	11	9%
Escherichia coli	10	8%
Acinetobacter baumanii	8	7%
Citrobacter sp	5	4%
Proteus sp	3	3%
No growth	17	14%
Bacterial flora		
Monomicrobial	62	60%
Polymicrobial	41	40%

Table 4: In vitro sensitivity of antimicrobial agents against Gram positive bacteria (n=72)

Antimicrobial agent	Sensitive organisms		
	Methicillin resistant Staphylococcus aureus (n=27) (%)	Coagulase Negative Staphylococci (n=21) (%)	Methicillin sensitive-Staphylococci aureus (n=11) (%)
Linezolid	27 (100%)	20 (96%)	11 (100%)
Vancomycin	26 (96%)	21 (100%)	9 (82%)
Clindamycin	22 (81%)	19 (90%)	11 (100%)
Gentamicin	17 (63%)	12 (57%)	8 (73%)
Cotrimoxazole	16 (59%)	11 (52%)	5 (45%)
Ciprofloxacin	14 (52%)	8 (38%)	7 (64%)
Erythromycin	11 (41%)	12 (57%)	8 (73%)
Penicillin	3 (11%)	4 (19%)	4 (36%)
Ampicillin	3 (11%)	1 (5%)	5 (45%)

limb threatening diabetic foot ulcers and are responsible for increased duration of hospitalization, cost of management, morbidity and mortality among diabetic patients.

For the treatment of DFI, the combination of debridement and antibiotics, coupled with good nutrition and diabetic control is paramount.[7] According to recommendations by the Infectious Disease Society of America (IDSA), empirical antimicrobial treatment should be initiated until the causative pathogens and their antibiotic susceptibility is known.[8]

Jain et al,[9] in their study had 81% males and 18% females. Higher male prevalence may be due to the higher level of outdoor physical activity with inadequate and improper feet care among males in comparison to females. The results were in concurrence with the findings of the current study. In a study by Mohite et al,[10] 53.80% of the cases had ulcers of Grade III and IV, whereas 12 patients had extensive gangrene (i.e., Grade V). 67.9% with majority of lesions located over sole area. These findings are consistent with present study.

In a recent Indian study of diabetic Foot Infections, bacterial etiology could be identified among 228 cases out of 253 (90%); single organism was isolated in 206 (90.3%) among which CONS and *S. aureus* being the most common, followed by *E. coli* and *Pseudomonas*. Similar results are noted in present study.[11] Otta S et al,.[12] had 62.2% of wound cultures showed monomicrobial flora and 27.1% had polymicrobial flora. In present study monomicrobial flora (61%) were more than with polymicrobial (39%). Gram positive isolates were more common than gram negative isolates in our study which is similar to the findings of Baba M et al,[13] and Malepati S et al.[14]

Staphylococcus aureus and *Pseudomonas aeruginosa* are important causative microorganisms in DFIs. The distributions of these causative organisms differ geographically and according to the illness duration, prior antibiotic use, and the relevance of nosocomial infections.[14] Also, the use of inappropriate antibiotics has become a problem for multi-drug resistant bacteria, making the selection of antibiotics difficult.[15]

Factors responsible for MDR may be frequent hospitalization, recent use of broad-spectrum antibiotics, inadequate surgical source reduction, chronic wounds, irrational use of antibiotics, and the transfer of resistance genes by transport means.[9] There is a recent emergence of the NDM metallo-beta-lactamase (MBL) encoding genes among different enterobacterial species and also in non-fermenters like *P. aeruginosa* and *Acinetobacter baumannii* in various parts of world including India.[16,17]

Most commonly encountered multi drug resistant organisms are methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin resistant Enterococci (VRE) and Gram-negative bacteria producing extended-spectrum beta-lactamases (ESBL), Metallo beta-lactamases (MBL). Large and deep ulcer, previous hospitalization and poor glycemic control are identified as some of the risk factors responsible for developing an infection with these drug-resistant microorganisms.[18]

Large, prospective, multicenter studies are required to assess the appropriate antibiotic regimen in diabetic foot ulcers and proper management of antibiotics must be implemented to decrease the incidence and development of multi drug resistant organisms.

CONCLUSION

Diabetic foot injuries &/or ulcer are a serious concern in patients with uncontrolled diabetics and require team approach for proper management. Early microbiological evaluation for bacteriological profile, the nature of the infection either monomicrobial or polymicrobial & antibiotic sensitivity testing can improve treatment outcome, reduces complications, morbidity as well as

multidrug resistance. Appropriate usage of antibiotics based on local antibiogram pattern can certainly help the clinician in reducing the burden of DFIs, which ultimately reduces the rate of amputations.

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REFERENCES

1. American Diabetes Association. Diagnosis and classification of diabetes mellitus. Diabetes Care 2009; 32:62-67.
   https://doi.org/10.2337/dc09-S062
2. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, et al. Infectious Diseases Society of America. 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2012; 54:e132-e173.
   https://doi.org/10.1093/cid/cis346
3. Aalaa M, Malazy OT, Sanjari M, Peimani M and Mohajeri- Tehrani MR. Nurses' role in diabetic foot prevention and care; a review. Journal of Diabetes & Metabolic Disorders. 2012; 11(1):24.
   https://doi.org/10.1186/2251-6581-11-24
4. Armstrong DG, Boulton AJM and Bus SA. Diabetic foot ulcers and their recurrence. N Engl J Med. 2017; 376:2367-2375.
   https://doi.org/10.1056/NEJMra1615439
5. Raspovic KM and Wukich DK. Self-reported quality of life and diabetic foot infections. J Foot Ankle Surg. 2014; 53:716-719.
   https://doi.org/10.1053/j.jfas.2014.06.011
6. Spichler A, Hurwitz BL, Armstrong DG and Lipsky BA. Microbiology of diabetic foot infections: from Louis Pasteur to 'crime scene investigation'. BMC Medicine. 2015; 13:2.
   https://doi.org/10.1186/s12916-014-0232-0
7. Son ST, Han S, Lee TY, Namgoong S and Dhong E. The microbiology of diabetic foot infections in Korea. J Wound Management Res. 2017; 13(1): 8-12.
   https://doi.org/10.22467/jwmr.2017.00108
8. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, Armstrong DG, et al. Infectious Disease Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis. 2012; 54(12): e132-e173.
   https://doi.org/10.1093/cid/cis346
9. Jain SK and Barman R. Bacteriological profile of diabetic foot ulcer with special reference to drug-resistant strains in a tertiary care center in North-East India. Indian J Endocr Metab. 2017; 21:688-694.
   https://doi.org/10.4103/ijem.IJEM_546_16
10. Mohite R, Karande GS and Chavan SK. Clinicobacteriological profile of diabetic foot ulcer among the patients attending rural tertiary health center. Int J Med Res Health Sci. 2014; 3:861-865.
    https://doi.org/10.5958/2319-5886.2014.00015.0
11. Vasanthan K, Vengadakrishnan K and Surendran P. Clinical Profile of Diabetic Foot Infections. Int J Sci Stud. 2018; 6(1):24-27.
12. Otta S, Debata NK and Swain B. Bacteriological profile of diabetic foot ulcers. CHRISMED J Health Res. 2019; 6:7-11.
    https://doi.org/10.4103/cjhr.cjhr_117_17
13. Baba M, Davis WA, Norman PE and Davis TME. Temporal changes in the prevalence and associates of foot ulceration in type 2 diabetes: the Fremantle Diabetes Study. J Diabetes Complications. 2015; 29(3):356-361.
    https://doi.org/10.1016/j.jdiacomp.2015.01.008
14. Malepati S, Vakamudi P, Kandati J and Sreeram S. Bacteriological study of diabetic foot ulcer according to Wagner's classification: a one-year study. Int Surg J. 2018; 5:98-104.
    https://doi.org/10.18203/2349-2902.isj20175534
15. Uckay I, Gariani K, Pataky Z and Lipsky BA. Diabetic foot infections: state-of-the-art. Diabetes Obes Metab. 2014; 16:305-316.
    https://doi.org/10.1111/dom.12190
16. Shanthi M, Sekar U, Kamalanathan A and Sekar B. Detection of New Delhi metallo beta lactamase-1 (NDM-1) carbapenemase in Pseudomonas aeruginosa in a single centrein southern India. Indian J Med Res. 2014; 140:546-550.
17. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, et al. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiologicalstudy. Lancet Infect Dis. 2010; 10:597-602.
    https://doi.org/10.1016/S1473-3099(10)70143-2
18. Gupta S, Attri A, Khanna S, Chander J and Mayankjayant. Outcome in Patients of Diabetic Foot Infection with Multidrug Resistant Organisms. J Foot Ankle Surg (Asia Pacific). 2018; 5(2):51-55.
    https://doi.org/10.5005/jp-journals-10040-1089

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SA-Concept and design of the study, prepared first draft of manuscript; **HM-**Coordination, review of literature and manuscript preparation; **HB-**Collection of the data and revision of manuscript.

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