Bacteriological Profile and their drug sensitivity profile in Diabetic Foot Ulcer, a report from a Tertiary Care Center

Abstract-

Aim: The present study was an attempt to evaluate the different microorganisms infecting the Diabetic foot ulcers (DFU) The aim of the current study was to compare the different bacteria infecting Diabetic Foot Ulcers. And to know the antibiotic susceptibility patterns among the isolates.

Study design- This is a Prospective observational study of patients treated at Tertiary health Care Centre, Pune.

Type of study- Prospective and observational hospital-based study

Period of study- From February 2021 to January 2022

Sample size- Tissue culture samples were collected from 100 patients.

Results: In our study, 81 patients were males and 19 were females.

In the present study, Grade I DFU was seen in 6 (6%) PWD, Grade II in 21 (21%), Grade III in 48 (48%), Grade IV in 21 (21%), and Grade V in 4 (4%).

In this study, 62 (62%) PWD cases had neuropathic conditions, 18 (18%) had neuropathic cases combined with sepsis, 11 (11%) had neuroischemic conditions, and 9 (9%) had neuroischemia plus sepsis. In this study, there were 29 (29%) polymicrobial cases, 64 (64%) monomicrobial cases, and in 7 (7%) cases, the culture was sterile.

Gram-positive bacterial growths were present in 41(41%) cases, whereas Gram-negative growth was seen in 59 (59%) cases.

The most common single bacterial growth was that of S. aureus (27%), followed by E. coli (20%), and Enterococcus spp. (15%).53% of the Gram-negative bacilli were Extended spectrum beta lactamase (ESBL) producers, 41% were Methicillin resistant staphylococcus aureus (MRSA), and 19% were Vancomycin resistant enterococci(VRE).[17,18]

Discussion : The majority of the PWD in this study were over 50 years old (63%). This could be a sign of comorbidities such neuropathy, peripheral vascular disease, and kidney illnesses being more common in this age group. A study by King et al. in 1998 also mentioned that the majority of people with diabetic foot were in 45–64 years

Higher male prevalence is comparable with study by Harrison and Lederberg. This might be because men engage in more outdoor physical activity than women, especially in hot, humid environments, with poor foot care

S. aureus was the single most frequent pathogen (26%) followed by E. coli (20%).

While GPC were more prevalent in Grades I and II, Gram-negative bacilli and mixed infections were more evident in Grades III and IV, suggesting that Gram-negative infections increase the severity and render patients more likely to require limb amputation.

Frequent hospitalization, frequent use of broad-spectrum antibiotics, insufficient surgical source reduction, chronic wounds, irrational use of antibiotics, and the transmission of resistance genes via transport methods are possible causes of MDR. Clinicians should use antibiotics judiciously, on time, and in sufficient amounts, and the relevant organizations should periodically monitor drug intake.

Conclusion: This study demonstrated that among the isolates from the DFUs, multidrug-resistant bacteria predominated. Determining the antibiotics for the empirical therapy of diabetic ulcers will be made easier with knowledge of the pattern of antibiotics resistance among the isolates. Thus, the likelihood of subsequent development of antibiotic resistance as well as the indiscriminate use of antibiotics can be reduced.

Keywords: Diabetic Foot ulcers, Bacteriological profile, Drug sensitivity profile

INTRODUCTION

Diabetic foot ulcer is a dangerous and common complication of diabetes mellitus (DM) that significantly increases the cost of treatment.[1]

Diabetic foot ulcers were found in 4.54% of patients newly diagnosed with type 2 diabetes mellitus in India; of these, 46.1% had neuropathic, 19.7% had ischemic, and 34.2% had neuroischemic foot ulcers.[2]

Infections, which account for 40% to 80% of instances of DFU morbidity and mortality, are most frequent complication in DFU.[3]

Poor microvascular circulation prevents phagocytic cells from reaching the infected location, which impairs the effectiveness of antibiotics in the infected tissue.[4]

Deep tissue infections rarely respond to antimicrobial therapy alone and generally require surgical procedures. Possible surgical interventions include incision and drainage, wound debridement, bone resection, tissue revascularisation and amputation.[31,32,33]

Infection causes the development of microthrombi, which aggravate ischemia, necrosis, and progressive gangrene necessitating limb amputation.[15]

Diabetes patients with severe soft tissue infections, significant osteomyelitis, extensive peripheral artery occlusion, extensive gangrene, and non-healing ulcers may necessitate lower-limb amputations[34,35,36]

DFUs are chronic in nature and patients with DFUs usually require several episodes of hospitalization. Patients are often exposed to several antibiotics which increase their risk of developing multidrug-resistant infection.[5]

Mostly, the diabetic foot infections (DFIs) are mixed bacterial infections, and the proper antibiotic selection is necessary for the treatment of these infections., based on the culture and the antimicrobial susceptibility testing results.[6]

AIM

The aim of the current study was to assess the various bacteria infecting the DFU and to know the antibiotic susceptibility patterns to the isolates.

METHODS

Inclusion criteria-

All the diabetic patients who attended the outpatient department of the study center with foot ulcer or infection.

Exclusion criteria-

Other foot ulcers and foot infection in persons without diabetes

The Institutional Ethical Committee's approval was obtained prior to conducting the study.

A clinical history was obtained in relation to the Patients with diabetes (PWD's) demographics, the length of the diabetes and foot condition, the type of diabetes treatment previously received, and the existence of any systemic disorders.

PWD underwent clinical evaluation as well, and the foot ulcers were rated using Wagner's grade (Wagner and Meggitt, 1970):

0 - No ulceration in a high-risk foot
1 - Superficial ulcer of skin or subcutaneous tissue
2 - Ulcers extend into tendon, bone, or capsule
3 - Deep ulcer with osteomyelitis or abscess
4 - Gangrene of toes or forefoot (localized gangrene)
5 - Extensive gangrene requiring a major amputation.[7]

Diabetic neuropathy is a result of chronic microvascular malfunction, oxidative stress,systemic inflammation all causing nerve damage.

Based on the presence of neuropathy, ischemia, and infection, ulcer foot type was determined. Investigations such as monofilament nerve conduction velocity testing, biothesiometry, and Doppler-based ankle brachial index estimation were carried out for this in addition to the clinical history and examination of PWD.[8]

After the debridement, tissue samples were taken.[9] Before obtaining a tissue sample, no antimicrobial or antiseptic agent was used to the wound. Empirical broad spectrum antibiotic coverage was started for every patient with DFU according to institutional protocol.

A deep tissue specimen (including fat, fascia, muscles, and bone) was also taken from the wound. The samples were put into sterile transport containers and delivered to the microbiology laboratory for aerobic microbial culture as soon as possible.

Anaerobic and fungal cultures were not performed for this study.

Most of the bacterial isolates were identified using VITEK 2 Compact system, and a few isolates were identified manually.A direct Gram□stained smear of the specimen was examined.

The specimens were inoculated onto blood agar, chocolate agar, Mac Conkey’s agar, and thioglycollate medium. The inoculated plates were incubated at a temperature of 37°C overnight, and the plates were examined for growth on the following day.

The organisms were identified on the basis of their Gram staining properties, and further analysis was done in VITEK® 2 Compact system (BioMérieux).[10]

Antibiotic susceptibility testing

A bacterial suspension was matched with McFarland standard of 0.5ml in 2.5 ml of a 0.45% sodium chloride solution with a VITEK® 2 DensiChek instrument (BioMérieux) with the incubation temperature kept at 35.5°C.

The isolates were subjected to a colorimetric measurement using a fresh optical reading head every 15 minutes for a maximum incubation time of 10 hours.

VITEK® 2 database version 4.01 was used to analyze the data for organism identification in kinetic mode after 2 h of incubation. The interpretations provided were then considered for the analysis.[11]

RESULTS

In the present study, out of 100 PWD, 38 patients were below 50 years and 62 patients were above 50 years.

In our study, 81 patients were males and 19 were females.

In the present study, Grade I DFU was seen in 6 (6%) PWD, Grade II in 21 (21%), Grade III in 48 (48%), Grade IV in 21 (21%), and Grade V in 4 (4%).

In this study, 62 (62%) PWD cases had neuropathic conditions, 18 (18%) had neuropathic cases combined with sepsis, 11 (11%) had neuroischemic conditions, and 9 (9%) had neuroischemia plus sepsis.

In this study, there were 29 (29%) polymicrobial cases, 64 (64%) monomicrobial cases, and in 7 (7%) cases, the culture was sterile.

Gram-positive bacterial growths were present in 41(41%) cases, whereas Gram-negative growth was seen in 59 (59%) cases.

The most common single bacterial growth was that of S. aureus (27%), followed by E. coli (20%), and Enterococcus spp. (15%).53% of the Gram-negative bacilli were Extended spectrum beta lactamase (ESBL) producers, 41% were Methicillin resistant staphylococcus aureus (MRSA), and 19% were Vancomycin resistant enterococci(VRE).

Bacterial sensitivity pattern is obtained as follow:

Antibiotics	Bacterial isolates along with	Sensitivity pattern (%)		
	Staphylococcus aureus	Enterococcus	Enterobacteriaceae	Pseudo
Ampicillin			11	
Amoxicillin-clavulanic acid			64	
Piperacillin-tazobactam			73	74
Cefalotin			24	
Ceftriaxone			56	
Cefoxitin			26	
Cefixime			11	
Ertapenem			76	
Ofloxacin			42	
Ticarcillin-clavulanic acid			14	67
Ceftazidime			64	72
Cefoperazone-sulbactam				68
Cefepime				74
Doripenem				87
Imipenem			89	72
Meropenem			84	70
Amikacin			90	90
Aztreonam				43
Gentamicin	83		89	66
Ciprofloxacin	73	74	65	67
Minocycline				54
Tigecycline				72
Trimethoprime-sulfamethoxa		47	39	22
Levofloxacin	74	68		68
Colistin				100
Oxacillin	72			
Erythromycin	78	70		
Clindamycin	71	58		
Linezolid	100	100		
Daptomycin	100	100		
Teicoplanin	84	89		
Vancomycin	100	67		
Benzylpenicillin	24	11		
Tetracycline		82		
Tigecycline	89	74		

In the present study, most of the Enterobacteriaceae culture isolates were sensitive to amikacin (90%), imipenem (89%), meropenem (84%)[28,29]

Most of the Pseudomonas culture isolates were sensitive to amikacin (90%), imipenem (72%), meropenem (70%).

Most of the Staphylococcus culture isolates were sensitive to linezolid (100%), daptomycin (100%), tigecycline (89%)

In our study, most of the Enterococcus culture isolates were sensitive to linezolid (100%),

daptomycin (100%), teicoplanin (89%), and tigecycline (74%)

DISCUSSION

The majority of the PWD in this study were over 50 years old (63%). This could be a sign of comorbidities such neuropathy, peripheral vascular disease, and kidney illnesses being more common in this age group. A study by King et al. in 1998 also mentioned that the majority of people with diabetic foot were in 45–64 years.[12]

Higher male prevalence is comparable with study by Harrison and Lederberg.[13] This might be because men engage in more outdoor physical activity than women, especially in hot, humid environments, with poor foot care.

Our study found that the majority of DFI patients reported having an advanced grade of infection, Wagner Grade III and above. This is frequently ascribed to the public's and medical professionals' lack of knowledge on foot care.[14]

S. aureus was the single most frequent pathogen (26%) followed by E. coli (20%). Other studies have also found the same (study by Abdulrazaq et al.)[18] In contrast, another study carried out by Ako□Nai et al. showed E. coli as the frequent bacterial pathogen, while P. aeruginosa was reported as the most common pathogen by Shankar et al Source of infection, use of antibiotic drug for treatment, sample collection method, and different types of infection can influence pathogen diversity in DFI.[19,20,21,22]

While GPC were more prevalent in Grades I and II, Gram-negative bacilli and mixed infections were more evident in Grades III and IV, suggesting that Gram-negative infections increase the severity and render patients more likely to require limb amputation.[24]

These days, the rising threat of MDR pathogens and related consequences in developing nations worries clinical microbiologists and doctors.[23] In the current investigation, 91 percent of the bacteria (VRE 33.33 percent, MRSA 48.14

percent, and ESBL 77.67 percent) were resistant to three or more antibiotics. In contrast to an Iranian study by Japoni et al, these rates are much higher . These isolate infections are more challenging to treat.[25.26]

Frequent hospitalization, frequent use of broad-spectrum antibiotics, insufficient surgical source reduction, chronic wounds, irrational use of antibiotics, and the transmission of resistance genes via transport methods are possible causes of MDR. Clinicians should use antibiotics judiciously, on time, and in sufficient amounts, and the relevant organizations should periodically monitor drug intake in order to improve the condition and lower the rate of amputation.

Clinicians should switch to using narrower spectrum therapy depending on culture report. To reduce infection sources, a sufficient and prompt surgical intervention is necessary.[27] These aid in lowering the excessive and careless use of antibiotics.

CONCLUSION

This study demonstrated that Gram-negative aerobes like S. aureus were the most frequent microbes found in diabetic foot ulcers.

In the DFI cases, monomicrobial infection was more prevalent than polymicrobial infection.[16]

MDR organisms were alarmingly prevalent in the PWD and in people with foot ulcers.

According to local sensitivity patterns ideal empirical antibiotics combination for Diabetic foot ulcers in our institution is Linezolid and Amikacin which is most effective in cohort of patients with presentation of infections associated with DFU.[30] In the present study, 91% of the bacteria were resistant to three or more antibiotics Thus, indiscriminate use of antibiotics and chances of subsequent development of antibiotic resistance can also be reduced with proper knowledge of antibiotics sensitivity

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