Int. J Med. Pharm. Res. (P-ISSN: 2958-3675 | E-ISSN: 2958-3683)
Available on: https://ijmpr.in/

International Journal
of Medical and
Pharmaceutical Research

ORGINAL ARTICLE **OPEN ACCESS**

Microbiological profiling of diabetic foot ulcers with antibiogram:A Research project under STS, ICMR

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Received: 25-11-2024
Accepted: 17-01-2025
Available online: 22-01-2025

ABSTRACT

Summary

Diabetic foot ulcers (DFUs) are a significant complication of Diabetes Mellitus, leading to reduced quality of life and even mortality in severe cases. Factors such as the immunocompromised state of the patient, hyperglycemia, and ischemia contribute to microbial colonization of DFUs, often resulting in severe complications like gangrene, osteomyelitis, and sepsis.

Despite advancements in treatment modalities, the standard treatment still involves the use of parenteral broad-spectrum antibiotics. This dependency contributes to the emergence of antibiotic resistance.

This prospective observational study aims to isolate and identify microorganisms from DFUs and determine their antibiotic susceptibility patterns. Swab samples collected from 15 diabetic patients undergo Gram staining, culture, and biomedical testing for microbial identification. This is followed by testing for antibiotic sensitivity to determine the pattern of antibiotic resistance in the isolated organisms.

Results indicate Escherichia coli as the most common organism isolated. Notably, all Staphylococcus isolated were methicillin-resistant (MRSA), and 60% of the E. coli isolates exhibit Extended-spectrum beta-lactamase (ESBL) production. Resistance of Proteus mirabilis to Tetracycline and Polymyxin B and that of Enterococcus faecalis to Tetracycline is also noteworthy.

In conclusion, this study shows the complexity of Diabetic foot ulcers and the increasing threat of antibiotic resistance in clinical practice.The findings indicate the importance of developing a more targeted and effective treatment strategy tailored to individual patients, there by reducing the risk of antimicrobial resistance, and enhancing patient outcomes in DFU management.

Keywords: Diabetic foot ulcer, Antibiogram, antibiotic resistance

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder, characterized by hyper glycaemia. Over time, this disease can progress, and cause multiple severe complications, involving various organsandorgan systems of the body. One such complication is diabetic foot ulcer (DFU).

DFUs as a complication can cause reduced quality of life due to deformity and disability. It may even cause death if left untreated. As the incidence of Diabetes increases in the urban world, research for better management of this condition becomes crucial.

Various elements present in a diabetic patient can lead to microbial colonization of the ulcer. These factors include[2]-

- Immunocompromised state of a diabetic individual leading to microbial in fections.
- Hyper glycaemia, which can favour microbial growth

- Ischaemia resulting in poor wound healing mechanisms. An open wound can easily be infected by environmental microbes

Diabetic foot infection is usually polymicrobial, but can also be caused by a single microorganism. Common microorganisms isolated from DFUs include bacteria such as Staphylococcus, Streptococcus, Enterobacteriaceae, pseudomonas, etc. Certain fungi, such as Candida, Aspergillus, Blastomyces, etc, may also be present but are less common.[3]

This microbial colonization of DFUs can lead to multiple severe complications such as gangrene of the foot, osteomyelitis, sepsis, permanent deformity, and foot amputation.[4] Not only does the condition pose cosmetic difficulty for the patient, but can also severely affect day-to-day function and lifespan.

Therefore, effective treatment of this condition is vital for patient well-being. The current treatment procedures involve parenteral broad-spectrum antibiotics, especially those that are active against Staphylococcus and Streptococcus, along with various wound-cleaningme thods to accelerate healing.[4]

This generalized treatment protocol is not based on any patient criteria. Additional data is required for better and more specific treatment. Moreover, continuous use of non-specific antibiotics can lead to antibiotic resistance in pathogens in case of long-term use. Therefore, there is a requirement for more patient-specific treatment regimens, with a lower risk of developing antibiotic resistance.

This research work is a prospective observational study that aims to provide data to support the need for a better management protocol for DFUs. The study is planned to determine the kinds of pathogens isolated from DFUs. This may help us devise better, more specific antibiotic regimens for cases of DFUs. The study will also help us recognize the current antibiotic resistance patterns in the given area, which will further lead to effective management of the condition.

METHODOLOGY

This is a prospective observational study conducted at sri shankaracharya medical college and associated hospital in India for duration of 2months for this study qualitative data (types of microorganisms isolated and antibiogram) was collected from total of 15subjects and Purpos ivesampling technique was used for this study.

- Inclusion criteria- subject must be a patient of diabetes mellitus with injury or diabetic foot ulcer (inpatient or outpatient) taking treatment at the hospital
- Exclusion criteria- patient with injury or trauma without any history of diabetes mellitus

Procedure

- 3swabs were collected from the ulcer or wound following all necessary as epticprecautions. One swab was used for Gramstaining. The second swab was used for culture inoculation on nutrient agar, blood agar, and Mc Conkey agar medium. The third swab was used for inoculation on Sabouraud Dextrose Agar (SDA) for the identification of fungi. Inoculation was done within 24 hours of sample collection.
- Gram staining- The sample from the first swab was used for Gram staining, followed by observation via light microscopy. Accordingly, the microorganisms was differentiated as Gram-positive and Gram-negative.
- Culture- The second swab used for inoculation on different standard culture media. Nutrient agar, Blood agar, and McConkey agar mediums were used for bacterial colonies. After inoculation, the agar plates were incubated at 37°C for at least 24 hours. Culture was observed for colony morphology and Gram differential staining done to confirm the organism
- The third swab was inoculated on SDA agar for fungal identification. 2 slants were inoculated. One slant was incubated at room temperature and the other in an incubator at 37°C. Culture morphology studied and LPCB Mount was done for the identification of fungal elements.
- Biochemical testing- Various standard biochemical tests like Catalase, coagulase,oxidase and bile esculin were carried out for the identification of gram positive organisms whereas oxidase, Indole, citrate, urease, MR, VP were carried out for identification of gram negative organisms.
- After identification of the pathogen spectrum isolated from DFU, an antibiogram was determined of all the organisms
- Procedure for determination of antibiogram-
  - Isolated organisms was inoculated in peptone water to form a bacterial suspension. The suspension was standardized to match turbidity of 0.5 McFarland
  - Antibiotic discswere placed on the surface of a freshly inoculated plate of Muller Hilton agar after doing lawn culture
  - Theplatewasincubatedat37°Cfor24hours
  - Plate was examined under transmitted light post incubation
  - Thezoneofinhibitionwasmeasuredinmillimeterswiththehelpofrulers/calipers

- CLSI2020 guidelines were followed for putting antibiotics and for interpretation of zone diameter.
- Accordingly, the isolated organism was termed susceptible, intermediate, or resistant

OBSERVATIONS AND RESULTS:

The patients belonged to the age group of 30-60 years with a predominance of males (73.33%) over females (26.66%) Microorganisms isolated from the DFU samples include-

1. E.coli
2. Staphylococcus aureus
3. Enter ococcus faecalis
4. Pseudomonas aeruginosa
5. Proteus mirabilis.

- The most common organism isolated from the samples was E. coli, with an occurrence of 27.7%.
- E. coli was closely followed by Staphylococcus Aureus, Enterococcus faecalis, and Pseudomonas aeruginosa with an occurrence of 22.2%.
- Proteus mirabil is was also isolated from one of the samples
- No fungal isolates were found.
- According to the antibiogram, 100% of the Staphylococcus isolated were methicillin- resistant **(MRSA)**
- 100% of the Staphylococcus isolates were found to be resistant to Cefoxitin, Erythromycin, Penicillin G, Co-trimoxazole, and Clindamycin. 75% were resistant to Levofloxacin, and 50% were resistant to Ampicillin
- No antibiotic resistance was observed in Pseudomonas aeruginosa isolates
- 60% of the E.coli isolates exhibit extended-spectrum beta-lactamase**(ESBL)** production.
- Additionally, E. coli isolates were also found to be resistant toAmpicillin (100%), Tetracycline (100%), Minocycline (20%), Cefuroxime (100%), Co-trimoxazole (80%), and Cefixime(80%)
- 100% of the Enterococcus faecalis isolates exhibit Cefoxitin resistance (intrinsic resistance). 50% show resistance to tetracycline
- Proteusmirabilis shows resistance to Tetracycline and Polymyxin B.

DISCUSSION

During this study, samples were collected from patients of DFUs. Most of these patients were between the ages of 35-60 years. A similar age distribution was observed in studies worldwide.[13]An evident predominance of males was observed with 73.33% of patients being males while 26.66% were females. A study conducted in Belgium showed a similar trend of male predominance with ulcers in males being more deeply infected.[14]This research work provides valuable insight into the microbiological aspect of DFUs. In this study, the identification of E.coli as the most prevalent organism, followed by Staphylococcus aureus, Enterococcus faecalis, and Pseudomonas aeruginosa, points towards the diverse microbiological aspects of DFUs. However, it is noteworthy that multiple studies suggest that the most common microorganism isolated from DFUs worldwide is Staphylococcusaureus[15], where as the most common in India is Pseudomonas aeruginosa.[16,17]Additionally, Proteus mirabilis was isolated from one of the samples in this study, this highlights the potential presence of less common pathogens in these wounds. All over the world, there have been multiple case studies documenting rare pathogens that cause DFUs.[18,19] These point towards the broad etiology of these ulcers. The presence of poly microbial cases in this study further emphasizes the complexity of DFUs. Furthermore, many cases of pathogenic biofilm production have also been observed.[20] These biofilms can promote synergistic behavior between different bacteria leading to the chronicity of infection and ultimately difficulty in treatment. Diabetic patients, due to their immunocompromised state are susceptible to pathogens that rarely cause infection in an otherwise healthy adult. Long-term treatment of these patients with broad-spectrum antibiotics can lead to resistant strains of pathogens infecting the wound. There is also a risk of other local and systemic infections being caused by these multi-drug resistant organisms (MDRO), like, osteomyelitis, sepsis, etc. which can further complicate treatment, and may even lead to death.It is very important to note the high rates of microbial resistance observed in this study. All Staphylococcus isolates demonstrate Mecithillin resistance (MRSA) and 80% of the E. coli isolates exhibit extended-spectrum beta-lactamase (ESBL) production. Similar trends of increasing antimicrobial resistance are seen all over the world in patients of DFUs.[21,22] These findings indicate that the rising incidence of antimicrobial resistance is a challenge for cliniciansal lover the world. The importance of antibiotic regimens guided by antibiograms to increase treatment efficacy and reduce further development of antimicrobial resistance is highlighted. Antibiotics should be used responsibly and with adequate precaution in clinical settings to not aggravate this crisis of anti-microbial resistance.

DFU patients have two times the risk of mortality as compared to non-ulcerated diabetic patients.[23] It is evident that the urgency of understanding complex conditions such as diabetes, DFUs, and antimicrobial resistance cannot be undermined. The results of this study along with the numerous other referenced studies indicated the need for modification and betterment of DFU management. There is undoubtedly a necessity for a more comprehensive andpatient-specific approach to treatment. In this way, the study calls for further development in the currently existing treatment protocol of DFUs.

CONCLUSION

In conclusion, this study calls for the development of a more patient-specific approach to the management of diabetic foot ulcers. A microbiological workup before starting a targeted treatment will go a long way in improving the quality of life of a diabetic patient. The study also calls for a multidisciplinary collaboration among clinicians and microbiologists to develop evidence-based strategies for DFU management. These strategies should include:

- Patient-specific antimicrobial therapy
- Wound care protocols
- Glycemic control measures
- Patient education

This will ensure a reduction in the risk of recurrent infections and will promote wound healing without any life-threatening complications. Further more, it can be concluded that antibiotic esistance is on therise, and is one of the most urgent problems faced by clinicians when it comes to treating bacterial infections. Thus, ongoing surveillance of microbial profiles and antibiotic resistance patterns is essential to guide the development of future therapeutic interventions. By addressing these challenges comprehensively, healthcare providers can strive to improve the outcomes and quality of life for individuals living with diabetic foot ulcers.

Acknowledgment: -

Authors would like to express sincere gratitude to ICMR for ICMR-STS programme under which this project was carried out. (Reference ID:2023-03664)

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