Original Article

Clinico-Microbiological Profile of Organisms Isolated from Diabetic Foot Ulcer at Tertiary Care Hospital, Gujarat

Dr. Nidhi Bhalodia[1*], Dr. Mihir Bhalodia[2], Dr.Sucheta Lakhani[3], Dr. Himani Pandya[4], Dr. Rachana Patel[4]

[1] Assistant Professor, Department of Microbiology, SBKSMI & RC Sumandeep Vidyapeeth, Piparia, Vadodara

[2] Assistant Professor, Department of Pathology, SBKSMI & RC Sumandeep vidyapeeth, Piparia, Vadodara

[3] Professor, Department of Microbiology, SBKSMI & RC Sumandeep Vidyapeeth, Piparia, Vadodara

[4] Associate Professor, Department of Microbiology, SBKSMI & RC Sumandeep Vidyapeeth, Piparia, Vadodara

ABSTRACT

Introduction: The individuals with diabetes have at least a 10-fold greater risk for soft tissue and bone infections of the foot than individuals without diabetes. The Indian diabetic population is expected to increase up to 57 million by the year 2025.

Aims and Objectives: To evaluate the bacteriological profile and antibiogram of diabetic foot ulcer.

Material and Methods: 150 Samples of diabetic foot ulcers were collected over a period of six months by using sterile swabs and they were processed as per the standard protocol. Pathogenic organisms were isolated, identified by biochemical tests. Antibiotic susceptibility testing was done by Kirby -Bauer disk diffusion method on Mueller Hinton Agar and results were interpreted as per Clinical and Laboratory Standards Institute guidelines.

Results: Bacterial etiology could be identified among 74 cases out of 150 (49.3%), among which Pseudomonas aeruginosa was the commonest (in 25 cases), followed by Klebsiella spp. (in 20 cases), Escherichia coli (in 13 cases), Proteus mirabilis (in 12 cases), Staphylococcus aureus (in 3 cases) and Enterococcus in 1 case.

Conclusion: Gram negative bacilli were more prevalent 70 out of 74 cases (94.5%) than gram positive cocci, 4 out of 74 cases (5.5%). In our study the commonest isolate was Pseudomonas aeruginosa (33.7%), followed by Klebsiella spp. (27.02%) and Escherichia coli (17.56%). Meropenem and Piperacillin/tazobactam are drug of choice in such cases.

Key Words: *Diabetic foot ulcer, Pseudomonas aeruginosa, ESBL*

*Corresponding Author
Dr. Nidhi Bhalodia*
Assistant Professor, Department of Microbiology, SBKSMI & RC Sumandeep Vidyapeeth, Piparia, Vadodara

INTRODUCTION

Diabetes mellitus is a chronic endocrine disorder leading to major complications like diabetic retinopathy, kidney damage, cardiovascular stroke and lower limb amputation [1]. Among low and middle income countries prevalence of diabetes is increasing at alarming rate [2]. The population of diabetic people raised from 108 million in 1980 to 422 million in 2014. The mortality rate also increased by 3% in between 2000 and 2019. WHO [3].

Diabetic foot ulcer is one of the serious complication of uncontrolled diabetes. It has been estimated that risk of developing diabetic foot ulcer has reached upto 68 per 1000 persons. The major factors contributing to development of diabetic foot ulcer are peripheral neuropathy, peripheral arterial disease and immunosuppression As a result microvascular circulation gets impaired which leads to poor penetration of antibiotics .This leads to heavy contamination of wound by bacterial pathogens resulting in formation of microthrombi [4, 5].

DFIs are caused by multidrug-resistant pathogens with the ability to form biofilm, which is an important virulence factor leading to treatment failure [6, 7]. Most common organism associated with diabetic foot infections are gram-positive bacteria such as Staphylococcus aureus, Enterococcus, and gram-negative organisms like Pseudomonas aeruginosa, Escherichia coli, Klebsiella species, Proteus species, etc., and anaerobes [8, 9 & 10]

The present study was carried out to evaluate the different microorganisms infecting the DFU and to know the antibiotic susceptibility patterns to the bacterial isolates. An increase in the population of multidrug organismsi. eextended spectrum beta-lactamase (ESBL)-producing organisms and methicillin-resistant Staphylococcus aureus

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(MRSA)among the DFU isolates was noticed. The knowledge of bacterial isolates from DFU is crucial for planning treatment with appropriate empirical antibiotics, reducing resistance pattern, and minimizing the cost of health care.

AIMS & OBJECTIVE
- To evaluate the bacteriological profile of diabetic foot infection.
- To evaluate the antimicrobial susceptibility pattern to formulate the policy of empirical antimicrobial therapy.

MATERIAL & METHOD
Total 150 pus samples of diabetic foot ulcer were collected over a period of six months from tertiary care Hospital Vadodara. Sterile swabs were used for collection of pus from the deeper portion of the ulcers. All swabs were collected before applying an antiseptic dressing to the wound and before starting treatment. After collection swabs were immediately transported to microbiology department and were subjected to gram staining and culture & sensitivity

The specimens were inoculated onto nutrient agar, chocolate agar and Mac Conkey's agar. The inoculated plates were incubated at 37°C for overnight and plates were examined for growth on next day. The organisms were identified on the basis of their Gram staining properties, colony morphology and their biochemical reactions.

Antibiotic susceptibility testing was done by Kirby -Bauer disk diffusion method on Mueller Hinton Agar and results were interpreted as per Clinical and Laboratory Standards Institute guidelines. Following antibiotics were used for gram negative isolates: Amikacin (30μg), Levofloxacin (5μg), Ceftazidime (30μg), Cefotaxime (30μg), Cefepime (30μg), Piperacillin/tazobactam(100/10μg), Meropenem (10 μg), Doxycycline (10 μg), Ceftazidime/clavulinic acid (30/10 μg), Cefotaxime /clavulinic acid.

Quality control strains
Escherichia coli ATCC 25922, Pseudomonas aeruginosa ATCC 27853, E. coli ATCC 35218, Enterococcus faecalis ATCC 29212, S. aureus ATCC 29213, and Enterococcus faecium ATCC 29212 were used as quality control strains during the evaluation of antibiotic sensitivity pattern.

A clinical history including duration of the diabetes and foot problem, the type of treatment for diabetes earlier received, and the presence of other systemic illnesses were taken.The diabetic foot ulcer were assessed according to Wagner's grade as follows:
- 0 - No ulceration formation
- 1 - Superficial ulceration of skin or subcutaneous tissue
- 2 - Ulcers extending to tendon, bone, or capsule
- 3 - Deep ulcer formation with osteomyelitis
- 4 - localized gangrene of foot
- 5 - Extensive gangrene requiring a major amputation

RESULT
One hundred and fifty samples were collected from patients with chronic diabetic foot ulcers. The study group comprised 89 male patients and 61 female patients, average age was between29–80 years. From these samples, 74 bacterial isolates were obtained. No polymicrobial infections were noted. Overall, 04 organisms (5.4%) were gram-positive and 70 organisms (94.5%) were gram-negative.

The diabetic foot ulcer were assessed by Wagner's grade according to which out of 150 , 9 patients were in Grade I, 32 patients in Grade II ,73 patients in Grade III, 30 patients in Grade IV and 6 patients in Grade V.

Pseudomonas aeruginosa(33.7%) and Klebsiella spp.(27.02%) were the most commonly isolated organisms followed by Escherichia coli (17.56%),Proteus mirabilis (16.2%),Staphylococcus aureus(4.05%)and Enterococcus faecalis(1.3%)

Pseudomonas aeruginosa showed maximum sensitivity to Piperacillin/tazobactam (96%) followed by Meropenem (92%),Ceftazidime/clavulinic acid (76%), Amikacin (60 %), Ceftazidime (52 %) and Levofloxacin (48 %)

Antibiotic sensitivity pattern of Klebsiella spp., Escherichia coli and Proteus mirabilis is depicted in Figure 1.

FIGURE 1

Name of antibiotic	Sensitivity %		
	Klebsiella spp.	*E.coli*	*Proteusmirabilis*
Amikacin	70%	76.9%	66.6%
Levofloxacin	60%	69.2%	75%
Meropenem	95%	100%	100%
Piperacillin/tazobactam	90%	92.30%	100%
Cefotaxime	20%	46.1%	50%
Doxycycline	10%	7.6%	0%
Cefotaxime/clavulinicacid	80%	84.6%	100%

Out of 3Staphylococcusaureusstrains isolated in the studytwo were Methicillin resistant strains. Staphylococcus aureus showed 100% sensitivity to Vancomycin, Linezolide and Gentamycin, 66.6% to Erythromycin and Clindamycin and 33.3% to Cefoxitin, Amoxycillin-clavulinic acid and co-trimoxazole. Enterococcus faecalis showed100% sensitivity to vancomycinand linezolid.

Among ESBL producers Klebsiellaspecies is most common, followed byEscherichiacoli and Proteusmirabilis.% ESBL is depicted in Figure 2.

FIGURE 2
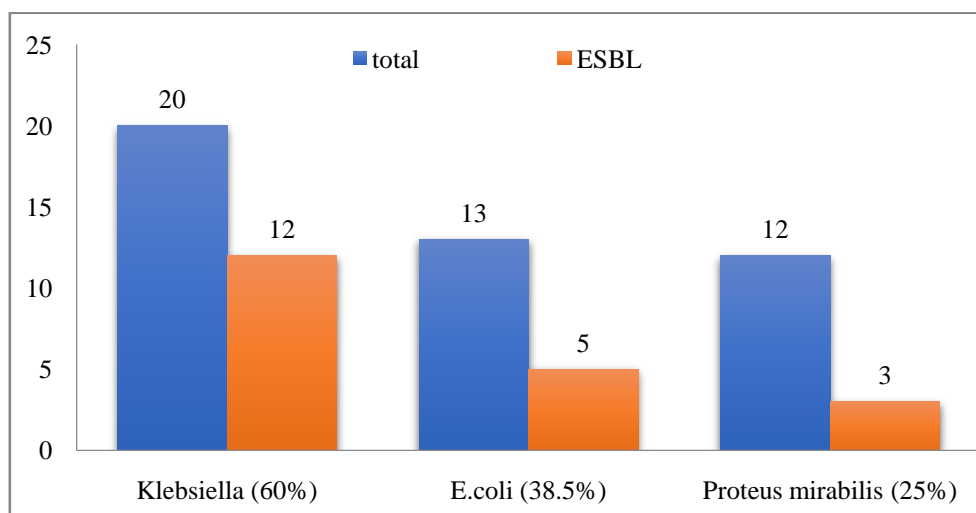
DISCUSSION

In this study Gram-negative microbes were identified with 94.5% prevalence. These findings correlated well with those of Manisha Jain et al [11] who reported that 82.8 % of the organisms were gram negative isolates in the study carried out at Tertiary Care Hospital at Ahmedabad, Gujarat.

Pseudomonas aeruginosawas predominant gram negativeisolate withprevalence of 33.7%. Jayashree Konar & Sanjeev Das [12] also reported Pseudomonasaeruginosa(31.34 %) asthe predominantgram negativeisolate.

In contrast, Abdul Jabbar Khaleel Ibrahim et al [13] reported Staphylococcusaureusas the predominant pathogen with(21.8%) prevalence in study carriedout inwestern India.

In our study Meropenem and Piperacillin/tazobactamwere drug of choicefor gram negative bacilli. This finding isin accordancewith astudy done by T. Deepa et al [23] in which gramnegative isolates showed 100% to carbapenems.

CONCLUSION

Gram negative bacilliwere moreprevalent 70 (94.5%) out of 74 casesthan grampositive cocci, 4 (5.5%)out of74 cases. In our studythe commonestisolate was Pseudomonasaeruginosa(33.7%), followed by Klebsiellaspp.(27.02%) and Escherichiacoli (17.56%).

In case ofdiabetic footinfection causedby gram negative organism **Piperacillin/ Tazobactam and Meropenem**are preferred first line of drug with more than90% sensitivity. In case of gram positiveisolates Vancomycin and Linezolide are drug of choice.

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Thus it is important to prepare an antibiogram of diabetic foot ulcer which will aid in timely management of patients and helps in preventing further complications.

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