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Bacteriological Profile and In-Vitro Antibiotic Sensitivity Pattern of Diabetic Foot Infections in a Tertiary Care Hospital

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Abstract **Original Research Article**

Foot infections are among the most common bacterial infections encountered in patients with diabetes mellitus in clinical practice. These infections and their sequelae are also the most common cause of disability and the reason for most hospital admissions among diabetic patients. The present study was a cross sectional study conducted in the Department of Microbiology in association with the Department of Medicine, Surgery and Orthopaedics, AIMSR, Bathinda, over a period of one year. Two sterile swabs were used to collect pus from each patient. One was used for direct microscopy by gram staining and other swab was used for culture onto MacConkey agar and Blood agar media. The following organism isolated were *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Acinetobacter baumannii*, and *Proteus mirabilis*. All gram positive organisms were found 100% sensitive to vancomycin and linezolid and maximum resistance was shown to erythromycin (67.50%) followed by penicillin G (57.50%). While gram negative organisms were found 100% sensitivity to colistin followed by polymyxin B (94.73%) and showed maximum resistance to ceftazidime (43.43%).
Keywords: Diabetic foot, MacConkey agar, Blood agar, *Pseudomonas aeroginosa*, *Staphylococcus aureus*, Antibiotic susceptibility testing, Mueller Hinton agar.

INTRODUCTION

Foot infections in persons with diabetes are an increasingly common problem and associated with potentially serious sequelae. According to the WHO and International Working Group on the Diabetic Foot, diabetic foot is defined as the foot of diabetic patients with ulceration, infection and/or destruction of the deep tissues, associated with neurological abnormalities and various degrees of peripheral vascular disease in the lower limb. It has been estimated that 15% of patients with type 2 DM have Diabetic Foot Ulcer (DFU) during their lifetime [1]. The impaired microvascular circulation in patients suffering from diabetic foot limits the access of phagocytes, favoring development of infection [2]. Ulcerations are prone to colonization by nearly every microorganism that comes in contact with their surface. Infection may be caused by pathogenic bacteria originating from the external environment as well as by bacteria forming physiological microflora of the skin. Usually ulcerations contain mixed flora, consisting of several strains of bacteria [3]. In diabetic foot disease, we should aim to diagnose infection at an early stage before it progresses towards deep infection and damages underlying tissues [4].

Due to advent of newer and sophisticated antibiotics the microbiological flora is constantly changing. The irrational use of antibiotics has led to immersion of multi drug resistance bacterial strains and disease complications in return. It is a well-known fact that the microbial drug resistance is a growing global problem. In gram negative bacteria, the most resistant pathogens are *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. The increasing trends observed resistance for all major anti-gram negative agents (β lactams, fluoroquinolones and aminoglycosides). Serious infection caused by gram positive bacteria such as MRSA is also difficult to treat. The detection of multi drug resistant isolates may further limit therapeutic options [5].

The importance of my study lies in the fact that DFU is a disease of multiple etiologies and its tendency for chronicity and dreaded complications calls for an earlier microbiological diagnosis and a prompt and effective treatment. The appropriate selection of antibiotics based on the antibiograms of isolates from diabetic foot infections is extremely critical for the proper management of these infections. This study has been planned to focus on microbial profile of DFU infections and correlate with the etiological diagnosis.

The knowledge of prevailing flora and their susceptibility to antimicrobials will guide the clinicians to prescribe an empirical regimen so that more specific management can be provided which will help in reducing resistance patterns, and minimize healthcare costs.

Aim and Objectives

AIM: To study microbiological profile and in-vitro antibiotic sensitivity pattern of diabetic foot infections in a tertiary care hospital.

Objectives

- To evaluate microbiological profile in cases of diabetic foot infections.
- To study the susceptibility pattern of bacterial isolates.

MATERIAL AND METHODS

After taking approval from Research Committee AIMSR, Bathinda, and Ethics Committee, Adesh University, Bathinda, present cross sectional study was conducted in the Department of Microbiology in collaboration with Department of Medicine, Department of Surgery and Department of Orthopaedics, AIMSR, Bathinda over a period of one year. An informed consent was obtained from every patient enrolled in the study. Patients fulfilling both clinical and microbiological criteria were included in the study.

All samples were processed by the standard microbiological techniques in the bacteriology laboratory of Microbiology department. Single use mini-tip sterile cotton swabs were used for sample collection and were transported in peptone water to maintain the swabs moist until being analyzed. All specimens were processed within 1 hour of collection. Two sterile swabs were collected from each patient. One swab was used for direct microscopy by gram staining, for the presence of epithelial cells, pus cells, and bacteria while the other swab was cultured aerobically on Blood agar and MacConkey agar media. The plates were then incubated overnight at 35-37°C for 24 hours [6].

Isolates were identified on the basis of colony characters like size, shape, surface, edges, margin, consistency, emulsifiability, opacity, color and any odour. Organisms were further confirmed on the basis of biochemical reactions and other specific confirmatory tests required for that particular organism. The following organisms were obtained from the study: *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, *Enterococcus faecium*, *Acinetobacter baumannii* and *Proteus mirabilis*.

Antimicrobial susceptibility testing was performed on Mueller Hinton Agar (MHA) by Kirby Bauer disk diffusion method. A suitable dilution (turbidity matching 0.5 McFarland standard) of peptone water growth of the test bacterium were inoculated on the surface of a solid medium (Mueller Hinton agar) as a lawn by spreading with a cotton swab. The predetermined antimicrobial discs were applied onto the surface of the inoculated agar. The plates were read after overnight incubation at 37^0C by measuring zone of inhibition around antibiotic discs as per CLSI (Clinical Laboratory Standards Institute) guidelines 2016[7].

RESULTS AND OBSERVATIONS

In present study, a total 97 patients were included. There was predominance of DFI in male patients (68.05%) with male: female ratio of 2.12:1. Maximum number of patients belonged to older age group i.e. 51-60 years (35.08%). 92 (94.84%) specimens were positive for culture and 05 (05.16%) specimens were sterile for growth. The most common predisposing factor found was poor diabetic control (80.41%), followed by neuropathy (69.07%), hypertension (40.20%). Out of 92 (94.84%) culture positive samples, 68 (73.91%) samples showed growth of single organism and 24 (26.08%) samples showed growth of mixed organisms (two organisms). Total 116 isolates were obtained from 92 culture positive samples. DFI showed a preponderance of gram-negative organisms (65.51%) over gram-positive organisms (34.49%). In present study, most common microorganism isolated was *Pseudomonas aeruginosa* (32.98%) followed by *Staphylococcus aureus* (29.89%), *Escherichia coli* (22.68%), *Klebsiella pneumoniae* (13.40), *Enterococcus faecalis* (7.21%), *Acinetobacter baumannii* (5.15%), *Enterococcus faecium* (4.12%) and *Proteus mirabilis* (4.12%) (Table 1,2). In present study, all isolates of *P. aeruginosa* were sensitive to colistin (100%), followed by 93.75% to polymyxin B and imipenem. More than 50% were resistant to ceftazidime and gentamicin. (Table 3) The antimicrobial susceptibility pattern of *S. aureus* and *Enterococcus* spp showed 100% sensitivity to vancomycin and linezolid while *S. aureus* showed maximum resistance to penicillin G (72.41%). (Table 4) *E. coli* and *K. pneumoniae* has shown 100% sensitivity to colistin and polymyxin B. (Table 5,6) *A. baumannii* showed 100% sensitivity to colistin followed by amikacin (80%) and polymyxin B (60%) while showed 100% resistance to piperacillin/tazobactam (Table 7) *P. mirabilis* showed 100% sensitivity to colistin, polymyxin B, imipenem, tigecycline and cefoperazone/sulbactam while to gentamicin organism showed 75% resistant (Table 8).

Table-1: Bacteriological profile

S No.	Organism isolated	Number	Percentage (%)
1	*Pseudomonas aeruginosa*	32	32.98
2	*Staphylococcus aureus*	29	29.89
3	*Escherichia coli*	22	22.68
4	*Klebsiella pneumonia*	13	13.40
5	*Enterococcus faecalis*	07	7.21
6	*Acinetobacter baumannii*	05	5.15
7	*Enterococcus faecium*	04	4.12
8	*Proteus mirabilis*	04	4.12
	Total	116	100

Table-2: Distribution of polymicrobial isolates

S. No	Mixed isolates	Frequency	Percentage (%)
1	*Escherichia coli + Staphylococcus aureus*	08	33.33
2	*Pseudomonas aeruginosa + Staphylococcus aureus*	07	29.16
3	*Escherichia coli + Klebseilla pneumonia*	05	20.84
4	*Enterococcus faecalis + Acinetobacter baumannii*	02	08.33
5	*Pseudomonas aeruginosa + Enterococcus faecalis*	02	08.33
	Total	24	100

Table-3: Antimicrobial susceptibilty testing of *Pseudomonas aeruginosa*

Drugs	Sensitivity		Resistance	
	Number	%	Number	%
Amikacin	26	81.25	06	18.75
Gentamicin	14	43.75	18	56.25
Cefepime	18	56.25	14	43.75
Cefuroxime	17	53.12	15	46.87
Ceftazidime	11	34.37	21	65.62
Cefoperazone	21	65.62	11	34.37
Amoxicillin/Clavulanate Potassium	27	84.37	05	15.62
Imipenem	30	93.75	02	06.25
Ciprofloxacin	20	62.50	12	37.50
Aztreonam	12	37.50	20	62.50
Piperacillin/Tazobactam	24	75.00	08	25.00
Trimethoprim/Sulfamethoxazole	24	75.00	08	25.00
Tigecycline	12	37.50	20	62.50
Polymyxin B	30	93.75	02	06.25
Colistin	32	100.00	00	00

Table-4: Antimicrobial susceptibility testing of *Staphylococcus aureus*

Drugs	Sensitivity		Resistance	
	Number	%	Number	%
Gentamicin	20	68.96	09	31.04
Cefoxitin	22	75.86	07	24.14
Ciprofloxacin	25	86.20	04	13.80
Levofloxacin	21	72.41	08	27.59
Penicillin G	08	27.58	21	72.41
Erythromycin	10	34.48	19	65.52
Clindamycin	17	58.62	12	41.38
Teicoplanin	24	82.75	05	17.25
Vancomycin	29	100.00	00	00
Chloramphenicol	22	75.86	07	24.14
Amoxicillin/Clavulanate Potassium	15	51.73	14	48.27
Linezolid	29	100.00	00	00
Trimethoprim/Sulfamethoxazole	14	48.27	15	51.73

Table-5: Antimicrobial susceptibilty testing of *Escherichia coli*

Drugs	Sensitivity		Resistance	
	Number	%	Number	%
Amikacin	16	72.72	06	27.28
Gentamicin	19	86.36	03	13.64
Cefuroxime	20	90.90	02	09.10
Cefepime	14	63.63	08	36.37
Ceftazidime	21	95.45	01	04.55
Cefoperazone	17	77.27	05	22.73
Imipenem	22	100.00	00	00
Ciprofloxacin	14	63.63	08	36.37
Piperacillin/Tazobactam	21	95.45	01	04.55
Amoxicillin/Clavulanate Potassium	22	100.00	00	00
Trimethoprim/Sulfamethoxazole	10	45.45	12	54.55
Tigecycline	22	100.00	00	00
Polymyxin B	22	100.00	00	00
Colistin	22	100.00	00	00

Table-6: Antimicrobial susceptibilty testing of *Klebsiella pneumoniae*

Drugs	Sensitivity		Resistance	
	Number	%	Number	%
Amikacin	12	92.30	01	07.70
Gentamicin	08	61.54	05	38.46
Cefuroxime	12	92.30	01	07.70
Cefepime	10	76.92	03	23.08
Ceftazidime	04	30.76	09	69.24
Cefoperazone	09	69.24	04	30.76
Imipenem	12	92.30	01	07.70
Ciprofloxacin	10	76.92	03	23.08
Piperacillin/Tazobactam	09	69.24	04	30.76
Amoxicillin/Clavulanate Potassium	12	92.30	01	07.70
Trimethoprim/Sulfamethoxazole	11	84.61	02	15.39
Tigecycline	05	38.46	08	61.54
Polymyxin B	13	100.00	00	00
Colistin	13	100.00	00	00

Table-7: Antimicrobial susceptibilty testing of *Acinetobacter baumannii*

Drugs	Sensitivity		Resistance	
	Number	%	Number	%
Amikacin	04	80.00	01	20.00
Gentamicin	03	60.00	02	40.00
Cefuroxime	02	40.00	03	60.00
Cefepime	03	60.00	02	40.00
Ceftazidime	03	60.00	02	40.00
Cefoperazone	02	40.00	03	60.00
Imipenem	03	60.00	02	40.00
Ciprofloxacin	02	40.00	03	60.00
Piperacillin/Tazobactam	00	00	05	100.00
Amoxicillin/Clavulanate Potassium	03	60.00	02	40.00
Trimethoprim/Sulfamethoxazole	02	40.00	03	60.00
Tigecycline	03	60.00	02	40.00
Polymyxin B	03	60.00	02	40.00
Colistin	05	100.00	00	00

Table-8: Antimicrobial susceptibilty testing of *Proteus mirabilis*

Drugs	Sensitivity		Resistance	
	Number	%	Number	%
Amikacin	04	100.00	00	00
Gentamicin	01	25.00	03	75.00
Cefuroxime	04	100.00	00	00
Cefepime	03	75.00	01	25.00
Ceftazidime	04	100.00	00	00
Cefoperazone	04	100.00	00	00
Imipenem	04	100.00	00	00
Ciprofloxacin	03	75.00	01	25.00
Piperacillin/Tazobactam	04	100.00	00	00
Amoxicillin/Clavulanate Potassium	03	75.00	01	25.00
Trimethoprim/Sulfamethoxazole	02	50.00	02	50.00
Tigecycline	04	100.00	00	00
Polymyxin B	04	100.00	00	00
Colistin	04	100.00	00	00

DISCUSSION

There was predominance of DFI in male patients (68.05%) with male: female ratio of 2.12:1. Similar findings were observed in a study by Saraswathy *et al.*[8] In contrast, study by Gupta *et al.* [9] found female (52%) predominance. The gender differences were statistically insignificant. The results obtained in our study can be attributed to higher level of outdoor physical activity in hot humid environment in males. Our work showed that the maximum number of patients belonged to older age group i.e. 51-60 years (35.08%). These findings were similar to the studies conducted by Patil *et al.*[10] who found 39.8% of the patients were between 51 to 60 years. The 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. Our findings showed that among 97 samples, 94.84% were culture positive and remaining 5.16% were sterile. Among the culture positive samples, 58.62% of culture growth was monomicrobial and 41.38% was polymicrobial. The low prevalence of polymicrobial infection may be attributable to the lack of severity of most infections and the low virulence of isolated organisms whereas in the study by Perim *et al.* [11] and Gupta *et al.* [9] DFU infection was predominantly polymicrobial. Microbiological evaluation of DFI showed a preponderance of gram-negative organisms (65.51%) over gram-positive organisms (34.49%), which is in accordance with earlier studies [12, 13]. In present study, most common microorganism isolated was *Pseudomonas aeruginosa* (32.98%) followed by *Staphylococcus aureus* (29.89%), *Escherichia coli* (22.68%), *Klebsiella pneumoniae* (13.40), *Enterococcus faecalis* (7.21%), *Acinetobacter baumannii* (5.15%), *Enterococcus faecium* (4.12%) and *Proteus mirabilis* (4.12%). In my study, polymicrobial growth was obtained in 24 cases. In 8 cases, *E. coli* along with *S. aureus* was isolated followed by 7 cases of *P. aeruginosa* and *S. aureus*, and 5 cases of *E. coli* along with *K. pneumoniae*. In a study conducted in South India [14], the most common combination was found to be of *Streptococcus pyogenes* and *S. aureus*. The difference may be due to diversity of organisms in different regions. All isolates of *P. aeruginosa* were sensitive to colistin (100%), followed by 93.75% to polymyxin B and imipenem and 75% to piperacillin/tazobactam. More than 50% were resistant to ceftazidime and gentamicin. Similar results were quoted in a study done by Shanmugam *et al.* [14] who also showed that *P. aeruginosa* was 100% sensitive to colistin and more than 50% resistant to gentamicin. The antimicrobial susceptibility pattern of *S. aureus* showed 100% sensitivity to vancomycin and linezolid followed by ciprofloxacin (86.20%) and teicoplanin (82.75%). This result was similar to that obtained by Raja *et al.*[15] Maximum resistance was seen to penicillin G (72.41%) and erythromycin (65.52%), which was also demonstrated by Ramani *et al.*[16] *E. coli* has shown 100% sensitivity to imipenem, colistin and polymyxin B followed by 95.45% to piperacillin/tazobactam. The antimicrobial susceptibility pattern of our study was in concordance with the study done by Shanmugam *et al.*[14] Antimicrobial susceptibility testing of *K. pneumoniae* showed 100% sensitivity to colistin and polymyxin B followed by 92.30% to imipenem. The maximum resistance was shown by ceftazidime (69.24%). This correlated with the findings of a study done in southern part of India [14]. *A. baumannii* showed 100% sensitivity to colistin followed by amikacin (80%) and polymyxin B (60%) while showed 100% resistance to piperacillin/tazobactam which was in accordance with studies by Ozer *et al.*[17], Raja *et al.*[15], and Gadepalli *et al.* [2]. *P. mirabilis* showed 100% sensitivity to colistin, polymyxin B, imipenem, tigecycline and cefoperazone/sulbactam while to gentamicin organism showed 75% resistant. A study by Tahawy[18] also revealed similar results.

Prevention of diabetic foot may include optimising metabolic control (regulating glucose levels); identification and screening of people at elevated risk for diabetic foot ulceration; and patient education to promote self-examination of foot and knowledge about foot care. Patients should be taught

routinely to inspect their feet. Control of footwear is also important as repeated trauma from tight shoes can be a triggering factor [19]. Treatment of diabetic foot can be challenging and prolonged; it requires antimicrobial drugs, topical dressings and in severe cases amputation may be required [20]. The choice of the initial antibiotic treatment depends on several factors such as the severity of the infection, whether the patient has received another antibiotic treatment for it, or whether the infection has been caused by a micro-organism that is known to be resistant to usual antibiotics (e.g. MRSA). The objective of antibiotic therapy is to stop the infection and ensure it does not spread [21].

CONCLUSION

The antimicrobial susceptibility data suggest that colistin, imipenem or piperacillin/tazobactam and vancomycin may be appropriate agents for empirical coverage. 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. Knowledge on the antibiotic susceptibility pattern of the isolates from diabetic foot infections is crucial for planning the appropriate empirical management of these cases, prior to getting the susceptibility reports from the laboratory.

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