



Original Research Article

Comparative bacteriological profile with antibiotic resistance pattern among diabetic and non-diabetic patients

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ABSTRACT

Background: Diabetes mellitus is a metabolic disorder which impedes the normal steps of the process of wound healing. It has higher risks of foot infection and postoperative wound infection that is polymicrobial with increased antibiotic resistance.

Objectives: A comparative study of bacteriological profile from the wound samples of diabetic and non-diabetic patients and to understand the relationship between bacterial load and high glycemic index among diabetic patients.

Materials and Methods: A cross sectional study involving 50 diabetic and 50 non-diabetic patients wound swab or pus samples collected under aseptic precautions. Results: Polymicrobial infection is mostly observed. Among the isolates, gram negative bacilli were about 55% and gram positive cocci were about 45%. The most common organism isolated was Staphylococcus aureus in diabetic and non-diabetic wound. In diabetic wound the predominant organism isolated was Staphylococcus aureus and Proteus mirabilis followed by Pseudomonas species. In non-diabetic wound, the predominant organism isolated was Staphylococcus aureus followed by Pseudomonas species. The Total Extended spectrum beta lactamase producer was about 76% whereas total Methicillin Resistant Staphylococcus aureus was 23%. In diabetic wound, glycemic index was poorly controlled in which fasting blood glucose level was > 150mg and post prandial blood glucose level was > 210mg among majority of cases.

Conclusion: Antibiotic susceptibility test screening showed gram positive cocci isolates to more sensitive towards Erythromycin, Vancomycin, Amikacin and gram negative bacilli isolates to be more sensitive for Amikacin, Gentamycin and Imipenem. Thus, early diagnosis of diabetic wound infection is required for the antimicrobial therapy.

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1. Introduction

Diabetes Mellitus a global disease, which was declared as an epidemic in developing countries where there is insulin deficiency causing increased blood glucose level. Approximately 347 million people are suffering from Diabetes Mellitus worldwide, which would be double by the year 2025.¹ With increased blood glucose level, there is always a lack of enough nutrients and oxygen to the energizing cells which reduces the normal functional activity of immune system and increases the process of inflammation in the body cells.¹ Reduced process of

wound healing leads to the peripheral arterial diseases and peripheral neuropathy causing reduced circulation and reduced oxygen supply, essential in the wound healing process.

Hyperglycemia facilitates growth of many organisms as well as colonization by bacterial and fungal pathogens.² Commonly isolated organisms include Staphylococcus aureus, Gram negative bacteria like Escherichia coli, Pseudomonas aeruginosa, Klebsiella species, Proteus species and anaerobic organisms. These infections are mostly polymicrobial with serious complications.³

On comparing the microbial profile of diabetic wound to that of non-diabetic wound, the severity of infection can

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be seen much aggravated among diabetic patients. This condition brings in recurrence of infection which further results in more comorbid conditions.⁴ Once an infection has developed in diabetic patients, it is difficult to treat because of impairment in microvascular circulation that limits phagocytic cell action and curtails the reach of antibiotics to the infected sites.⁵

Early diagnosis of diabetic wound infection is required for the antimicrobial therapy. This study is expected to generate valuable information, which will be helpful in the management and prevention of diabetic infection in our population and will guide clinicians in the management of such condition with appropriate as well as judicious use of antimicrobial therapy.

2. Materials and Methods

The present study was a cross-sectional type of study, carried out in the Department of Microbiology for a period of two months between October to November 2018. The study was started after getting institutional ethics clearance from the College. A total of 100 wound swab or pus samples were collected from 50 diabetic patients and 50 non-diabetic patients of any age group for the study. To avoid contamination, wound and tissue debris were thoroughly cleaned with sterile normal saline followed by gentle rubbing of the wound site with 70% alcohol prior to swabbing the pus sample.

Samples were collected in sterile screw-capped containers and were transported to the laboratory immediately. If it was wound swab, two swabs were collected per patient where one was used for microscopy (Gram's staining) and the other for routine conventional culture method. Blood was also collected for biochemical analysis for the estimation of glycemic index.

Samples were properly labeled and transported to the laboratory for further investigation. In further methodology, the identification of aerobic bacteria and detection of its antimicrobial susceptibility was performed by Kirby Bauer disk diffusion method as per the Clinical and Laboratory Standards Institute (CLSI) guidelines 2017.⁶ Extended spectrum beta-lactamases (ESBL) positive organisms were screened by observing an increase ≥ 5 mm in zone diameter with ceftazidime/clavulanate versus its zone size when tested with ceftazidime disc alone.⁷ Resistance to methicillin was tested using the cefoxitin disc (30 μ g) by disc diffusion method. An inhibition zone diameter of ≤ 21 mm in case of *Staphylococcus aureus* and ≤ 24 mm for coagulase negative *Staphylococcus* was considered as methicillin resistant organisms.

3. Results

A total of 100 samples accounting for 50 diabetic and 50 non-diabetic were included for the study. The study population

represented 70% male whereas 30% as female patients. The mean age of the patients was 52.31 years ranging from 11 to 99 years. All patients presented with ulcers which were graded 0-5 in the Wagner classification and majority belonged to Grade 2 (i.e., deep ulcer, penetrating down to ligaments and muscles, but no bone involvement or abscess formation).^{8,9}

Among the total 100 samples, 55% were wound swabs and 45% accounted for the pus samples received from the surgery department. All the samples showed bacterial growth of aerobic bacteria. The distribution of bacteriological profile is shown in the Tables 1 and 2.

Our study showed polymicrobial distribution of infection and it was found to be about 32% among the total samples. The most common organism isolated was *Staphylococcus aureus* in both diabetic and non-diabetic wound samples. There was 45% of gram positive isolates whereas 55% were identified as gram negative isolates. Among all the isolates, *Staphylococcus aureus* was the most frequent pathogen (26%), followed by *Pseudomonas aeruginosa* (16%) and *Proteus mirabilis* (14%).

Among the gram positive organisms, *Staphylococcus aureus* (57.8%) was most frequent in which 6% were found to be methicillin resistant *Staphylococcus aureus* (MRSA). The second commonest pathogen in gram positive organism was *Enterococcus* spp (17.8%) followed by coagulase negative staphylococci (13.3%) which are mainly recognized as normal commensals.

Pseudomonas aeruginosa (29%) was the predominant pathogen isolated among the gram negative organisms followed by *Proteus mirabilis* (25.4%) and *Escherichia coli* (14.5%).

In-vitro antibiotic susceptibility pattern are depicted in Table 3 & 4. All gram positive isolates were sensitive towards vancomycin and linezolid. Also, Fusidic acid was found to be efficient against all *Staphylococcus* species including the methicillin resistant isolates. Among the gram negative, most of the isolates were sensitive to Amikacin, Cotrimoxazole and Imipenem. Only one isolate of *Acinetobacter* spp (1.8%) showed resistance towards Imipenem.

The total Methicillin Resistant *Staphylococcus aureus* was 6%, in which both diabetic and non-diabetic wound was about 3% each. The total extended spectrum beta lactamases organism in our study was about 23% among which 16% was diabetic whereas only 7% was non-diabetic.

On comparison among diabetic wound, the glycaemic index was poorly controlled wherein fasting blood glucose level was found to be >150 mg/dL in 37 patients and postprandial blood glucose level was about >210 mg/dL in 41 patients. These patients also found to have polymicrobial growth of microbial pathogens which were resistant to most of the antibiotics.

Table 1: Distribution of bacterial isolates among the clinical samples (N=100)

Organism	N (%)
Gram positive bacteria	
Staphylococcus aureus (MS) ^a	6
Staphylococcus aureus (MR) ^b	20
Enterococcus spp	8
Coagulase negative Staphylococcus	6
Streptococcus spp	5
Gram Negative bacteria	
Pseudomonas aeruginosa	16
Proteus mirabilis	14
Escherichia coli	8
Klebsiella pneumoniae	6
Acinetobacter spp	6
Atypical E.coli	5
Total	100 (100%)

^aMS- methicillin- sensitive; ^bMR- methicillin resistant

Table 2: Percentage distribution of organisms among the clinical isolates

Organism	DM-wound	Non-DM wound
Gram positive cocci		
Staphylococcus aureus	14%	12%
Enterococci	3%	5%
Streptococcus	3%	2%
Staphylococcus (coagulase –ve)	1%	5%
Gram negative bacilli		
Proteus mirabilis (ESBL)	10%	4%
Pseudomonas aeruginosa	8%	8%
E.coli	6%	2%
Klebsiella pneumoniae (ESBL)	5%	1%
Acinetobacter	4%	2%
Atypical E.coli	4%	1%

Table 3: In-vitro susceptibility pattern of Gram positive organisms (N= 45) to various antimicrobials (%)

Organism		ERY	CIP	COT	AZIT	CD	VAN	LZ	FA
Staphylococcus aureus (MS) ^a	3	80	54	54	48	61	100	100	100
Staphylococcus aureus (MR) ^b	0	54	26	50	52	29	100	100	100
Enterococcus spp	12	29	81	74	27	75	100	100	100
Staphylococcus (coagulase negative)	0	83	75	39	48	81	100	100	100
Streptococcus spp	4	65	69	49	51	29	100	100	100

^aMS- methicillin- sensitive; ^bMR- methicillin resistant

P-penicillin; ERY- erythromycin; CIP- ciprofloxacin; COT- cotrimoxazole; AZIT-azithromycin, CD- clindamycin, VAN- vancomycin, LZ-lincolid; FA- fusidic acid

Table 4: In-vitro susceptibility pattern of Gram negative organisms (N= 55) to various antimicrobials (%)

Organism	AK	CTX	CIP	COT	AMC	IPM	ATM	CES
<i>Pseudomonas aeruginosa</i>	65	25	54	57	10	100	89	89
<i>Proteus mirabilis</i>	59	65	26	95	47	100	59	69
<i>Escherichia coli</i>	85	57	81	86	52	100	69	100
<i>Klebsiella pneumoniae</i>	87	45	75	69	41	100	74	67
<i>Acinetobacter spp</i>	69	29	69	74	25	98	63	80
Atypical <i>E.coli</i>	85	58	45	68	39	100	71	75

^aMS- methicillin- sensitive; ^bMR- methicillin resistant

AK-amikacin; CTX- cefotaxime; CIP- ciprofloxacin; COT- cotrimoxazole; AMC-amoxicillin/ clavulanic acid, IPM- imipenem, ATM- aztreonam, CES-cefoperazone/sulbactam

4. Discussion

In our study, diabetics mostly in the aged category were more prone and susceptible for the diabetic foot infections. This was found to be in agreement with the study from Bangladesh which too reports most of their patients being in the older category.¹⁰ The mean age of the patients was 52.31 years which was similar to the 55 mean age of the subjects reported from the Bangladesh study.¹⁰

Isolation of only aerobic pathogens were found in both the diabetic and non-diabetic categories which included gram positive and gram negative organisms owing to the aerobic incubation conditions followed in the laboratory whereas other studies showed isolation of anaerobic pathogens also in the diabetic foot infections.¹¹

Among the isolated pathogens *Staphylococcus aureus* was the commonest gram positive bacteria and in the gram negative category *Pseudomonas aeruginosa* was the frequently isolated microbial pathogen in the diabetic foot ulcer patients as well as in non-diabetic patients. These findings were similar to the many other Indian and international studies carried out among the diabetic foot infections.^{12,13}

Diabetic foot infections are considered to be polymicrobial in nature. This polymicrobial infection rate was comparable to the other studies where polymicrobial isolation of pathogens were more when compared to the monomicrobial organisms.^{14,15} Also, preponderance of the organisms isolated is gram positive followed by gram negative and anaerobic pathogens.^{16–18}

Like other reported studies, our findings state presence of antibiotic resistant organisms like methicillin resistance and ESBL producers more common among the diabetic people on comparing with non-diabetic patients.¹⁸

The polymicrobial nature of the diabetic foot infections reveals indirect relationship between the occurrence of bacterial infections and increased duration of the diabetic foot ulcer which results in many complications like amputation of the affected parts.

Effective antimicrobial usage is prerequisite in the control and care of the diabetic foot infections. Antibiotic

susceptibility pattern of the commonly isolated organisms are low and none of the isolates prove 100% efficient in treating the infections. Also, like other reported studies there is presence of multiple antibiotic resistance in the isolated microorganisms.^{19,20} This low susceptibility for antimicrobial agents attributes for the extensive usage of the available treatment options without the judicious thinking.

Though, *Pseudomonas* species show the highest rate of isolation among diabetic as well as non-diabetic like other studies, we also recommend adequate care like avoiding moisture frequent wound dressings etc. to eradicate this pathogen.²¹ Also, overall incidence of *Staphylococcus spp* is considered to be more as stated in other similar studies among diabetic as well as among non-diabetic patients.²²

Our findings are comparable to a recent study done suggesting more chronic and complicated diabetic foot infections are by gram negative pathogens predominantly.²³

5. Limitation of the Study

Comprehensive antimicrobial coverage in treating diabetic foot infections need to be studied on a larger group of patients. Isolation of anaerobic pathogens and their role in the pathogenesis of diabetic foot infections need to be emphasized.

6. Conclusion

It is concluded through our study that polymicrobial infections are more prevalent among diabetic patients when compared to non-diabetic group of patients. Hence, early diagnosis of diabetic wound infections with proper therapy and care is essential in order to avoid complications and deep seeded systemic infections among diabetic patients.

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Self.

9. Conflict of Interest

None.

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