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A Study of Microbial Profile and Drug Resistance Pattern in the Patients with Diabetic Foot at a Tertiary Health Care Center

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Abstract

Introduction: Diabetic Foot Ulcer (DFU) is a serious and common complication of Diabetes Mellitus (DM). India has been called "the diabetes capital of the world." The most common cause of morbidity and mortality in DFU is infections. Diabetic foot wounds are commonly infected, and infection leads to the formation of microthrombi causing further ischemia, necrosis, and progressive gangrene. These types of situations necessitate limb amputation. Thus, accurate diagnosis of the causative organism is essential for the management of these cases. **Aims and Objectives**: To Study microbial profile as well as drug resistance pattern in the patients having diabetic foot at a tertiary care center. **Methodology**: Approval from institutional ethical committee was obtained. 62 patients of diabetic foot were selected for the present study over 1 year. Wound discharge culture and antibiotic sensitivity testing were carried out using standard microbiological procedures. **Result**: In the study carried out it was observed that the majority of the patients fell under the age group consisting of those >60. The most common organism was Staphylococcus followed by Streptococci, Enterococcus. Gram positive organisms were most commonly sensitive to Vancomycin. Gram negative organisms were susceptible to Piperacillin-Tazobactum. Maximum resistance was found to be for Gentamycin. **Conclusion**: From the study it can be conclusively noticed that majority patients belonged to the age group consisting of >60 Yrs. Gram positive organisms showed highest sensitivity to Vancomycin. In case of Gram negative organism's antibiotic with highest sensitivity was Piperacillin-Tazobactam. Gentamicin was the drug with highest resistance. This resistance pattern to various pathogens in our study will be helpful in treatment of Diabetic foot in future.

Keywords: Diabetic Foot, Diabetic Foot Ulcer (DFU), Drug Resistance Pattern

1. Introduction

Diabetic Foot Ulcer (DFU) is a serious and common complication of Diabetes Mellitus (DM). India is said to be "the diabetes capital of the world". Further it is estimated that about 41 million in India are affected by the disease. "Every fifth diabetic in the world is an Indian"[1]. In the United States, presently DM affects about 8.3% of the population. Further about 79 million individuals are said to be prediabetes[2]. Among persons with diabetes (PWD), 12%–25% have a risk of developing a foot ulcer during their lifetime[3–5]. The most common cause of morbidity and mortality in DFU is infections, which are seen in 40%–80% of the cases[6]. Diabetic neuropathy and microischemia are the two main risk factors that cause DFU[7]. Impaired microvascular circulation limits the

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access of phagocytic cells to infected area, and this results in poor concentration of antibiotics in infected tissue[8]. Hence, diabetic foot wounds frequently get infected. Due to infection, there may be subsequent formation of microthrombi. This may in turn lead to development of ischemia, necrosis, and in some cases progression to gangrene. These types of situations necessitate limb amputation. Thus, accurate diagnosis of the causative organism is essential for the management of these cases. It is predicted that by 2030 diabetes mellitus may afflict up to 79.4 million individuals in India[4]. Persons with DM are more predisposed to skin and soft tissue infections such as folliculitis, furunculosis, and subcutaneous abscesses[9]. This in turn indicates there will be increase in number of Diabetes Foot Ulcer cases in near future.

2. Aims and Objectives

To Study microbial profile as well as drug resistance pattern in the patients having diabetic foot at a tertiary care center.

3. Methodology

Approval from institutional ethical committee was obtained. After which cross-sectional study consisting of patients with diabetic foot was carried out. This was done at a tertiary health care center over a period of one year. Beginning from January 2017 till January 2018. For this study patients admitted for the diabetic foot in the surgical ward were selected. During one-year period there were 62 patients admitted for the diabetic foot. In this study demographic details of the patients were obtained. Collection of pus discharge from the wound was done under all aseptic precautions. It was then sent to Microbiological lab for the microbiological identification and culture sensitivity. Further antimicrobial susceptibility of the concerned bacterial isolates was determined. This was done using the disk diffusion method. For which guidelines followed were that of the CLSI[10]. The ANOVA test was used for statistical analysis and was calculated by using SPSS 19 version of concerned software.

4. Observation and Results

Table 1 shows the age distribution of the study participants. Around 30% patients were from age group more than 60 years followed by 50-60 years.

Proportion of males was found higher (59.68%) than females (40.32%) (Table 2).

Table 1. Distribution according to age of the patients

Age	Number	Percentage (%)
20-30	3	4.84 %
30-40	8	12.90 %
40-50	15	24.19 %
50-60	17	27.42 %
>60	19	30.65 %
Total	62	100.00 %

Table 2. Distribution of the patients according to the sex

Sex	Number	Percentage (%)
Male	37	59.68 %
Female	25	40.32 %
Total	62	100.00 %

Table 3. Microbial profile based grouping of patients

Staphylococcus	20	32.26%
Streptococci	14	22.58%
Enterococcus spp.	13	20.97%
Pseudomonas	11	17.74%
Escherichia coli	11	17.74%
Klebsiella	9	14.52%
Proteus	3	4.84%
Providencia	2	3.22%
Acinetobacter	1	1.61%

Table 3 shows the microbial profile of diabetic foot found on culture. Most commonly isolated organisms were Staphylococcus, Streptococcus and Enterococcus. Among 62 participants, more than one organism was isolated.

Table 4 shows the antibiotic sensitivity and resistance pattern. Among Gram positive organisms Staphylococcus were most sensitive (least resistant) to Vancomycin followed by Rifampicin, Sulfamethoxazole/ Trimethoprim, Clindamycin, Ampicillin, Cloxacillin, Amoxicillin-Clavulanic acid, Erythromycin, Fusidic acid, Tetracycline, Penicillin in decreasing order of sensitivity. Similarly, Streptococcuss was most sensitive to Vancomycin Rifampicin, Ampicillin followed by Amoxicillin-Clavulanic acid, Erythromycin, Sulfamethoxazole/ Trimethoprim, Clindamycin, Fusidic acid, Cloxacillin, Gentamycin, Tetracycline, Penicillin. Enterococcus ssp. were most sensitive to Vancomycin, Ampicillin followed by Clindamycin, Rifampicin, Gentamycin, Cloxacillin, Amoxicillin-Clavulanic acid, Tetracycline, Erythromycin, Fusidic acid, Sulfamethoxazole/Trimethoprim, Penicillin. In case of Gram negative organisms, Escherichia Coli were most sensitive (least resistant) to Piperacillin-Tazobactam, Imipenem, Amikacin followed by Piperacillin, Cefotaxime, Cefuroxime, Sulfamethoxazole/ Trimethoprim, Ceftazidime, Gentamicin, Ciprofloxacin. Similarly, Pseudomonas were most sensitive (least resistant) to Piperacillin-Tazobactam followed by Imipenem, Amikacin, Cefotaxime, Amoxicillin- Clavulanic acid, Cefuroxime, Sulphamethoxazole/Trimethoprim, Gentamicin, Ceftazidime, Ciprofloxacin. Klebsiella

Table 4. Distribution based on resistance pattern of gram positive organism

	Gram Positive Organism (% Resistant)					
	Staphylococcus (20)		**Streptococcus (14)**		**Enterococcus spp. (13)**	
Ampicillin	6	30 %	1	7.14 %	0	0
Clindamycin	5	25 %	3	21.42 %	2	15.38 %
Cloxacillin	6	30 %	4	28.57 %	4	30.76 %
Amoxicillin-Clavulanic Acid	6	30 %	2	14.28 %	4	30.76 %
Erytrothromycin	6	30 %	2	14.28 %	5	38.46 %
Fusidic acid	6	30 %	3	21.42 %	6	46.15 %
Gentamycin	5	25 %	4	28.57 %	3	23.07 %
Penicillin	13	65 %	7	50.00 %	8	61.53 %
Rifampicin	4	20 %	1	7.14 %	2	15.38 %
Sulfamethoxazole/ Trimethoprim	5	25 %	2	14.28 %	7	53.84 %
Tetracycline	7	35 %	5	35.71 %	4	30.76 %
Vancomycin	3	15 %	1	7.14 %	0	0

Table 5. Distribution of the patients as per the resistance pattern of gram-negative organism

Antimicrobial agents	**Gram-negative organisms (% Resistant)**							
	Escherichia coli (11)		**Pseudomonas (11)**		**Klebsiella(9)**		**Proteus (3)**	
		(%)		(%)		(%)		(%)
Amikacin	1	9.09 %	2	18.18 %	2	22.22 %	0	0.00
Amoxicillin- Clavulanic acid	6	54.54 %	3	27.27 %	3	33.33 %	2	66.67 %

Table 5 Continued

Cefotaxime	3	27.27 %	2	18.18 %	1	11.11 %	1	33.33 %
Ceftazidime	4	36.36 %	4	36.36 %	2	22.22 %	0	0.00
Cefuroxime	3	27.27 %	3	27.27 %	3	33.33 %	1	33.33 %
Ciprofloxacin	6	54.54 %	5	45.45 %	4	44.44 %	2	66.67 %
Gentamicin	5	45.45 %	4	36.36 %	4	44.44 %	2	66.67 %
Imipenem	1	9.09 %	2	18.18 %	0	0.00	0	0.00
Piperacillin	2	18.18 %	2	18.18 %	1	11.11 %	1	33.33 %
Piperacillin—tazobactam	1	9.09 %	1	9.09 %	0	0.00	0	0.00
Sulfamethoxazole/ Trimethoprim	3	27.27 %	3	27.27 %	6	66.66 %	1	33.33 %

were most sensitive (least resistant) to Piperacillin-Tazobactam followed by Imipenem, Cefotaxime, Piperacillin, Amikacin, Ceftazidime, Amoxicillin-Clavulanic acid, Cefuroxime, Ciprofloxacin, Gentamicin, Sulphamethoxazole/Trimethoprim. Lastly Pseudomonas were most sensitive (least resistant) to Piperacillin-Tazobactam, Imipenem, Ceftazidime, Amikacin followed by Cefotaxime, Cefuroxime, Piperacillin, Sulfamethoxazole/ Trimethoprim, Amoxicillin-Clavulanic acid, Ciprofloxacin, Gentamicin (table 5).

5. Discussion

Diabetic Foot Infections (DFIs) are commonly seen as consequence of Diabetes Mellitus. They lead to significant morbidities. This leads to significant increase in hospital stay in terms of number of days. They also account for highest number of proximate amputation which is due to some cause not related to trauma[11,12]. There are number of studies on the bacteriology of diabetic foot infections. Findings of these studies indicate that these infections could be either due to single microorganism or multiple microorganisms. It must be noted that infections caused by multiple organisms are more severe in nature. Infecting organisms are commonly aerobic Gram positive cocci, Gram-negative bacilli and anaerobes[13]. Proper management of DFIs can help reduce the severity. It may also help to decrease the duration of hospitalization. All these also lead to decrease in need for amputation. Hence early recognition of the condition, appropriate intervention, study of microorganism profile, starting desired antibiotic therapy at correct point in time will help improve the clinical outcome[14].

In this study it was observed that large bulk of the patients belonged to group of >60 years of age. Number of patients increasing as we move to higher age group. This trend of increase in number of cases with increase in age was statistically significant (ANOVA; $F=45.43$, $P<0.006$). The majority of the patients were patients were Male. The most common organism was Staphylococcus followed by Streptococci, Enterococcus. There were on an average 1.4 pathogens per lesion. When we look at resistance pattern, among Gram positive organisms Staphylococcus were most sensitive (least resistant) to Vancomycin followed by Rifampicin, Sulfamethoxazole/Trimethoprim, Clindamycin, Ampicillin, Cloxacillin, Amoxicillin-Clavulanic acid, Erythromycin, Fusidic acid, Tetracycline,

Penicillin in decreasing order of sensitivity. Similarly, Streptococcuss was most sensitive to Vancomycin Rifampicin, Ampicillin followed by Amoxicillin-Clavulanic acid, Erythromycin, Sulfamethoxazole/Trimethoprim, Clindamycin, Fusidic acid, Cloxacillin, Gentamycin, Tetracycline, Penicillin. *Enterococcus ssp.* were most sensitive to Vancomycin, Ampicillin followed by Clindamycin, Rifampicin, Gentamycin, Cloxacillin, Amoxicillin-Clavulanic acid, Tetracycline, Erythromycin, Fusidic acid, Sulfamethoxazole/Trimethoprim, Penicillin. In case of Gram negative organisms, *Escherichia coli* were most sensitive (least resistant) to Piperacillin-Tazobactam, Imipenem, Amikacin followed by Piperacillin, Cefotaxime, Cefuroxime, Sulfamethoxazole/Trimethoprim, Ceftazidime, Gentamicin, Ciprofloxacin. Similarly, Pseudomonas were most sensitive (least resistant) to Piperacillin-Tazobactam followed by Imipenem, Amikacin and thereafter to Cefotaxime, Amoxicillin-Clavulanic acid, Cefuroxime, Sulphamethoxazole/Trimethoprim, Gentamicin, Ceftazidime, Ciprofloxacin. Klebsiella were most sensitive (least resistant) to Piperacillin-Tazobactam followed by Imipenem, Cefotaxime, Piperacillin, Amikacin, Ceftazidime, Amoxicillin-Clavulanic acid, Cefuroxime, Ciprofloxacin, Gentamicin, Sulphamethoxazole/Trimethoprim. Lastly Now we take a look at some similar studies, Khalifa Al Benwan *et al.*[15] they studied 440 patients altogether. From these patients overall 777 pathogens were obtained. Thus on an average 1.8 pathogens were seen per lesion. Majority was constituted by Gram-negative pathogens, which accounted for 51.2%. This was followed by Gram-positive pathogens. These accounted for 32.3%. At last anaerobes were seen in 15.3% cases. Polymicrobial infection were seen in almost ¾th of the patients. The isolated organisms ranged from Enterobacteriaceae family to *Pseudomonas aeruginosa*. It also included Staphylococcus, followed by anaerobic Gram-negative organisms and *Enterococcus* spp. Vancomycin was seen to be most effective in treatment for Gram-positive bacteria. On the other hand, imipenem, piperacillin—tazobactam and amikacin were highly potent inmanaging Gram-negative bacteria. In summary, infections are more common among diabetic patients. There was tendency of finding multiple organisms per wound. Large bulk of isolates were resistant to multiple drugs.

Sudhir K Jain *et al.*[16] – this study included 150 cases of diabetic foot ulcers. From these 185 isolates were obtained. The persons included in study ranged from as low as 35 years to as high as 80 years. Large bulk of patients were from 60-65 years of age. Among the isolates, Gram-negative bacilli were seen in larger number of cases followed by Gram-positive cocci. *Staphylococcus* spp. was most commonly seen organism. This was followed by *Escherichia coli*. Thereafter, it was *Enterococcus* spp. when we look at antibiotic sensitivity production of beta lactamase was seen in some cases, around 53% of Gram negative bacilli. Some cases of methicillin resistance in *Staphylococcus aureus* were seen.

Diwan Mahmood Khan *et al.*[17] found that maximum number of patients were in the age group 45 to 64 years and male predominant compared to female. The most commonly found isolate was *Pseudomonas aeruginosa* (39.6%), *Escherichia coli* (17.46%), Acinetobacter species (15.41%), Proteus species (12.47%), and Klebsiella species (9.75%) respectively. Antimicrobial susceptibility of the Gram negative bacteria was also studied. ESBL producing most common bacteria was Klebsiella species and Citrobacter species in this study than *Escherichia coli* and Enterobacter species.

Sanjith Saseedharan *et al.*[18] - this study consisted of approximately 60% males and 40% females. The study consisted of 261 patients. Samples were obtained from these cases. Large bulk of samples contained more than one organism. Among the organisms seen, gram negative pathogens were seen in majority cases. Among all isolates, seven isolates were fungal. *Staphylococcus aureus* was most common bacteria isolated. This was followed by *Pseudomonas aeruginosa*. Around ¼th of *Staphylococci* were resistant to methicillin.

6. Conclusion

From this study it can be concluded that large bulk of patients were more than 60 years of age. The increasing number of cases with increase in age was statistically significant. The majority of the patients were male. When we consider Gram positive organisms antibiotic with highest sensitivity was Vancomycin succeeded by Rifampicin, Ampicillin, Amoxicillin-clavulanic acid, Clindamycin, Gentamicin, in decreasing order of sensitivity. Penicillin was the drug with highest resistance.

In case of Gram negative organisms antibiotic with highest sensitivity was Piperacillin- Tazobactam succeeded by Imipenem, Amikacin, Cefotaxime, cefuroxime, ceftazidime and Sulphamethoxazole/ Trimethoprim sequentially. Ciprofloxacin, Gentamicin were the drugs with highest resistance.

This resistance pattern to various pathogens in our study will facilitate our selection of antibiotics in treatment of Diabetic foot in future. Thus it will guide us in the selecting drug of choice and enable rational use of antibiotcs. It will also prevent development of antibiotic resistance.

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