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Frequency of Different Microorganisms Isolated from the Diabetic Foot Ulcers and their Antibiotic Susceptibility Pattern in a tertiary care hospital Lahore: A Retrospective cohort study

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Abstract

The main prospective of this study to detect the frequency of different microorganisms isolated from Diabetic Foot Ulcer (DFUs) and also their antibiotic susceptibility pattern. On the basis of purposive sampling technique the medical record of 356 patients was collected who have Diabetic foot ulcer and visited the Sakina Begum Institute of Diabetes and Endocrine Research Lahore (SIDER) from May 2021 to May 2022. Only those samples were included in this study whose bacterial growth was identified through microbiological media and further antimicrobial sensitivity was confirmed by disc diffusion method. About 54.5% of diabetic foot ulcer patients were tested positive for infections by microbes; among which 62% samples had single organism growth and 37% had polymicrobial growth. 34% of the isolated microbes were gram positive while 66% were gram negative. Staphylococcus aureus was the most prevalent isolated organism because it showed the strong resistant pattern against cefotaxime, cefuroxime and ampicillin but vancomycin have the 58% sensitivity for gram positive bacteria. Most of the isolates showed resistance to common antibiotics, so appropriate antibiotic administration is necessary to reduce the resistance pattern in DFUs and needs to be timely treated to avoid from further infection.

Keywords: Diabetes, Foot ulcer, Infections, Micro-organisms, Sensitivity, Antibiotics

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1. INTRODUCTION

Diabetes mellitus (DM) is widely recognized as an emerging epidemic that impacts almost every country, age group, and economy across the world[1]. Diabetic foot ulcer (DFU) is among the most common complications of diabetes mellitus that is estimated in approximately 15-20% in patients with DM[2]. Diabetic foot ulceration is characterized by impaired wound healing in the lower extremities which mostly causes hospitalization and accounts for about 85% of all non-traumatic lower extremity amputations in these patients[3]. The global

prevalence of Diabetic Foot Ulcer is 6.3%. In Pakistan, the studies indicate that the prevalence of diabetes foot ulcers ranged from 4.0% to 10.0%[4]. Risk factors for foot ulcers in patients with diabetes include: previous lower extremity amputation, peripheral neuropathy, and duration of diabetes for more than 10 years, peripheral vascular disease, patients of rural areas, diabetic nephropathy patients who are on dialysis, poor glycemic control, tobacco chewing and smoking[5,6].The developmental pathway toward diabetic foot ulceration is multifactorial[7] and the patho-physiology of diabetic foot ulcers has neuropathic, vascular, and immune system components, which all show a base relationship with the hyperglycemic state of diabetes[8]. Diabetic patients have an altered function of polymorphonuclear cells and impaired phagocytosis, chemotaxis, and bactericidal activity (related to both non oxidative and oxidative mechanisms), which are more evident in the presence of hyperglycemia[9]. Patients may not notice foot wounds because of decreased peripheral sensation. Because the blood supply required in healing a diabetic foot ulcer is greater than that needed to maintain intact skin[5]. It is also estimated that 45% to 60% of all ulcerations in patients with diabetes are mainly due to neuropathy, while 45% of the ulcers are due to combined neuropathic and ischemic factors[10].Sometimes these wounds are colonized with microorganisms that may lead to a state of clinical infection, which in turn cause a large amount of morbidity[11].

Diabetic foot infections can drive the clinical spectrum from superficial cellulitis (mild infection) to chronic osteomyelitis (severe infection). Host–microorganism interaction is very crucial in determining progression of DFU[12]. Diabetic foot infections are mostly polymicrobial, consisting of both Gram positive and Gram-negative aerobic bacteria. Hyperglycemia produces oxidative stress on nerve cells and leads to neuropathy. Glycosylation of nerve cell proteins causes nerve cell dysfunction, leading to further ischemia. These cellular changes result in motor, autonomic, and sensory complications of neuropathic foot ulcers. Peripheral arterial disease is a known cause of diabetic foot. Biofilm formation is an important pathophysiological step in diabetic foot ulcers, it plays a main role in the disease progression and chronicity of the lesion, the development of antibiotic resistance, and makes wound healing difficult to treat. 80% of lower-limb amputations in diabetic patients are preceded by biofilm infected foot ulceration[9]. This study aims to differentiate the DFU with microbial infections from the non-infectious ones and to check their antibiotic susceptibility pattern for timely and proper treatment plan.

2. MATERIALS AND METHODS

2.1 Studied Area

This retrospective cohort study was conducted in Sakina Begum Institute of Diabetes and Endocrine Research (SIDER), Shalamar Hospital Lahore, Pakistan from 27-07-2022 to 02-02- 2023. The study was permitted by the Institutional Ethics Committee (SSAHS-IRB/AL/43/2022) and was in accordance with the declaration of World Medical Association (WMA) made at Helsinki.

2.2 Data Collection

The data of 356 patients with DFU were included on the basis of study Performa which consists of the personal information, organisms isolated and antibiotics susceptibility pattern. Diabetic foot ulcer patients of both genders (male and female) were included in this study while DFU patients who was already on antibiotic treatment were excluded.

2.3 Isolation and Characterization

The organism were isolated on Blood Agar, Chocolate Agar, MacConkey agar and Bile Esculin Agar as well as further microscopic and biochemical characterization of the isolated organism was done by gram staining, Catalase test, DNase test and through analytical profile index (API).

2.4 Antimicrobial susceptibility

Antimicrobial susceptibility was tested by using the disc diffusion method and the antibiotic panel included commonly used antimicrobials such as cefuroxime, ceftriaxone, co-trimoxazole, ampicillin, amikacin, imipenem, cefotaxime, ciprofloxacin, erythromycin, gentamicin, vancomycin and piperacillin/tazobactam. Susceptibility of antibiotics was determined by measuring the zone of inhibition in according to the Clinical Laboratory Standard Institute Guidelines of 2020 (CLSI).

2.5 Statistical Analysis

The data was entered and analyzed by SPSS 25. Frequencies were calculated for qualitative data (such as gender and antibiotic susceptibility pattern) whereas mean and standard deviation was computed for quantitative variables (such as age, gender).

3. RESULTS AND DISCUSSIONS

Diabetic foot ulcers (DFUs) are a serious and common problem in patients with diabetes mellitus and constitute one of the major causes of lower extremity amputation.[12] This study aims to differentiate the DFU with microbial infections from the non-infectious ones and also their antibiotic susceptibility pattern for timely and proper treatment plan. Out of 356 positive patients 199(55.9%) were male and 157(44.1%) were female in which 162 samples were reported negative for any bacterial growth whereas 194 samples were reported positive. According to the positive results of 194 patients the 116 were male and 78 were female which further distributed for mon-microbial growth and poly-microbial growth. 69 males showed mon-microbial growth and 47 males have poly-microbial growth as well as if we talk about the growth distribution among female then 53 have mono growth and 25 have poly microbial growth. A study conducted by Shahi SK and his colleagues also showed that the rate of DFU was higher in males as compared to females[6]. A systemic review by Zhang P et al also suggested that diabetic foot ulceration was more prevalent in male diabetic patients (4.5%) than female patients (3.5%)[13]. The results of the current study show that among all the samples, 54.5% were reported positive for bacterial growth which is compatible with the findings of the study carried out by Ahmadishooli A et al in Southern Iran, which revealed that about half of DFU often become infectious[2].

According to microbiological evaluation of DFI revealed that 122 (34.3%) samples were monomicrobial infection and 72 (20.2%) were with polymicrobial infection and the mean age of patients with non-infectious DFU was 50 years while the mean age of mono-microbial infections and poly-microbial infection was 48 years and 53 years respectively. Of the 194 culture positive samples, 62% samples had single organism growth and 37% had polymicrobial growth. This result is inconsistent with a study carried out in Karachi which reported polymicrobial infections in 83% of patients[14]. In the present study, among the 278 microbes isolated, 66% were gram negative bacteria and 34% were gram positive. Some previous studies yielded similar results where gram negative bacteria were more predominant[15,16,17,18] whereas some studies showed the predominance of gram positive bacteria[19,20].

Out of 356 samples, 278 microbes were isolated in which gram positive bacteria were found in 94 samples (34%) whereas gram negative bacteria were found in 184 samples (66%). The most prevalent bacterium was *Staphylococcus aureus* (22.5%) followed by *Pseudomonas spp.* (12.6%), *Escherichia coli* (11.8%), *Klebsiella spp*. (9%) *Proteus spp.* (6.7%), *Citrobacter spp.* (4.8%), *Acinetobacter baumannii* (3.4), *Enterococcus spp.* (2.8), *Providentia spp*. (1.7%), *Morganella spp. (*1.1%), *Streptococcus pyogenes* (1.1%), *Serratia spp*. (0.6%).The antibiotic susceptibility pattern of isolated gram-positive bacteria showed that vancomycin had the highest sensitivity rate (64%), followed by gentamicin (59%) and imipenem (56%). The drug with highest resistance was ampicillin (13%) followed by cefotaxime (22%) and cefuroxime (22%). In concordance to previous studies, the results of this study revealed that *Staphylococcus aureus* was the most prevalent organism accounting for 22.5% of isolates followed by *Pseudomonas* (12.6%)[21,22]. A study conducted by Atlaw et al revealed the similar results up to first three prevalent bacteria i.e. *Staphylococcus aureus* ,*Pseudomonas* and *E*. *coli*[23].This variation in microorganism cultures may be due to difference of geographical areas and variance in risk factors in developing foot ulcers. According to our study, the results revealed that both gram positive and gram-negative bacteria showed higher sensitivity to vancomycin, gentamicin and imipenem. The sensitivity pattern of gram-negative bacteria was similar to gram positive bacteria but with a higher sensitivity rate i.e., Vancomycin (67%), gentamicin (61%), imipenem (59%). Whereas the resistance pattern was found a bit altered with cefuroxime (13%) being the most resistant followed closely by cefotaxime (16%) and ampicillin (17%). The senstivity and resistance pattern of different drugs for gram positive and gram negative bacteria is illustrated in table No. 1 and 2. Sekhar and his colleagues reported that bacterial isolates were 100% sensitive to cotrimoxazole and

totally resistant to ciprofloxacin[24]. Ogba and his coworkers carried out a study in Nigeria which reported that gram-positive isolates showed higher susceptibility to erythromycin, followed by amoxicillin. The gram-negative isolates were more susceptible to ciprofloxacin, followed by amoxicillin[25]. In another study by Qadir AN et al imipenem was an effective antibiotic against isolated microorganisms[26]. The differences in results reported by various studies indicate that antibiotic pattern is inconsistent in patients of diabetic foot ulcers. Antibiotic resistance can be explained by multiple courses of antibiotics which is common in patients with DFUs.

Table 1: Anti-bacterial susceptibility pattern of gram-negative bacteria.

Antibiotics		***Acinetobacter baumannii***	***Citrobacter***	***Escherichia coli***	***Klebsiella spp.***	***Morganella***	***Providentia spp.***	***Proteus spp.***	***Pseudomonas spp.***	***Serratia spp***
Ceftriaxone	**S**	4(36.4%)	5(29.4%)	7(16.7%)	8(25%)	1(25%)	2(40%)	9(39.1%)	15(33.3%)	1(50%)
	R	7(63.6%)	12(70.6%)	35(83.3%)	24(75%)	3(75%)	3(60%)	14(60.9%)	30(66.7%)	1(50%)
Cefuroxime	**S**	1(9.1%)	1(5.9%)	6(14.3%)	5(15.6%)	0(0%)	1(20%)	4(17.4%)	5(11.1%)	0.00%
	R	10(90.9%)	16(94.1%)	36(85.7%)	27(84.4%)	4(100%)	4(80%)	19(82.6%)	40(88.9%)	2(100%)
Co Trimoxazole	**S**	1(9.1%)	4(23.5%)	11(26.2%)	9(28.1%)	1(25%)	4(80%)	6(26.1%)	9(20%)	0.00%
	R	10(90.9%)	13(76.5%)	31(73.8%)	23(71.9%)	3(75%)	1(20%)	17(73.9%)	36(80%)	2(100%)
Cefotaxime	**S**	1(9.1%)	1(5.9%)	6(14.3%)	3(9.4%)	2(50%)	1(20%)	7(30.4%)	8(17.8%)	0.00%
	R	10(90.9%)	16(94.1%)	36(85.7%)	29(90.6%)	2(50%)	4(80%)	16(69.6%)	37(82.2%)	2(100%)
Ciprofloxacin	**S**	4(36.4%)	6(35.3%)	12(28.6%)	10(31.3%)	1(25%)	3(60%)	8(34.8%)	20(44.4%)	0.00%

	R	7(63.6%)	11(64.7%)	30(71.4%)	22(68.8%)	3(75%)	2(40%)	15(65.2%)	25(55.6%)	2(100%)
Imipenem	**S**	7(63.6%)	11(64.7%)	24(57.1%)	15(46.9%)	2(50%)	3(60%)	16(69.6%)	26(57.8%)	2(100%)
	R	4(36.4%)	6(35.3%)	18(42.9%)	17(53.1%)	2(50%)	2(40%)	7(30.4%)	19(42.2%)	0.00%
Amikacin	**S**	4(36.4%)	6(35.3%)	30(71.4%)	16(50%)	2(50%)	5(100%)	15(65.2%)	20(44.4%)	1(50%)
	R	7(63.6%)	11(64.7%)	12(28.6%)	16(50%)	2(50%)	0(0%)	8(34.8%)	25(55.6%)	1(50%)
Ampicillin	**S**	2(18.2%)	5(29.4%)	8(19%)	4(12.5%)	1(25%)	0(0%)	4(17.4%)	6(13.3%)	0.00%
	R	9(81.8%)	12(70.6%)	34(81%)	28(87.5%)	3(75%)	5(100%)	19(82.6%)	39(86.7%)	2(100%)
Erythromycin	**S**	2(18.2%)	4(23.5%)	9(21.4%)	5(15.6%)	1(25%)	2(40%)	3(13%)	11(24.4%)	0.00%
	R	9(81.8%)	13(76.5%)	33(78.6%)	27(84.4%)	3(75%)	3(60%)	20(87%)	34(75.6%)	2(100%)
Vancomycin	**S**	6(54.5%)	10(58.8%)	26(61.9%)	23(71.9%)	3(75%)	4(80%)	16(69.6%)	28(62.2%)	1(50%)
	R	5(45.5%)	7(41.2%)	16(38.1%)	9(28.1%)	1(25%)	1(20%)	7(30.4%)	17(37.8%)	1(50%)
Gentamicin	**S**	8(72.7%)	9(52.9%)	26(61.9%)	19(59.4%	1(25%)	3(60%)	11(47.8	32(71.1%)	1(50%)

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	R	3(27.3%)	8(47.1%)	16(38.1%)	13(40.6%)	3(75%)	2(40%)	12(52.2%)	13(28.9%)	1(50%)
Piperacillin/ Tazobactam	**S**	5(45.5%)	6(35.3%)	14(33.3%)	12(37.5%)	1(25%)	2(40%)	11(47.8%)	17(37.8%)	0.00%
	R	6(54.5%)	11(64.7%)	28(66.7%)	20(62.5%)	3(75%)	3(60%)	12(52.2%)	28(62.2%)	2(100%)

S: Sensitivity R: Resistance

Table 2: Anti-bacterial susceptibility pattern of gram-positive bacteria.

Antibiotics		***Enterococcus***	***Staphylococcus aureus***	***Streptococcus pyogenes***
Ceftriaxone	**S**	2(20%)	25(32.5%)	2(50%)
	R	8(80%)	52(67.5%)	2(50%)
Cefuroxime	**S**	4(40%)	14(18.2%)	2(50%)
	R	6(60%)	63(81.8%)	2(50%)
Co Trimoxazole	**S**	2(20%)	23(29.9%)	1(25%)
	R	8(80%)	54(70.1%)	3(75%)
Cefotaxime	**S**	2(20%)	17(22.1%)	1(25%)
	R	8(80%)	60(77.9%)	3(75%)
Ciprofloxacin	**S**	1(10%)	26(33.8%)	3(75%)
	R	9(90%0	51(66.2%)	1(25%)
Imipenem	**S**	6(60%)	44(57.1%)	1(25%)
	R	4(40%)	33(42.9%)	3(75%)
Amikacin	**S**	4(40%)	39(50.6%)	2(50%)
	R	6(60%)	38(49.4%)	2(50%)
Ampicillin	**S**	1(10%)	11(14.3%)	1(25%)
	R	9(90%0	66(85.7%)	3(75%)
Erythromycin	**S**	3(30%)	18(23.4%)	1(25%)
	R	7(70%)	59(76.6%)	3(75%)
Vancomycin	**S**	6(60%)	49(63.6%)	3(75%)
	R	4(40%)	28(36.4%)	1(25%)
Gentamicin	**S**	7(70%)	46(59.7%)	1(25%)
	R	3(30%)	31(40.3%)	3(75%)
Piperacillin/ Tazobactam	**S**	3(30%)	42(54.5%)	2(50%)
	R	7(70%)	35(45.5%)	2(50%)

4. CONCLUSIONS

Most of the diabetic foot ulcers become infected with microorganisms. Our study showed that monomicrobial infections were more common in patients with DFIs. *Pseudomonas* was most prevalent micro-organism among gram negative bacteria while *Staphylococcus aureus* was prevalent among gram positive bacteria for DFI. In addition, vancomycin, gentamycin and imipenem were effective antibiotics against isolated microorganisms. Evaluating the antibiotic susceptibility patterns of microbes from diabetic foot infections is essential as it would be a laed mark for the better treatment of DFI. This study's limitations include the lack of molecular investigation of drug resistance patterns. Molecular techniques can improve microbial characterization and antibiotic therapy. This study was based on in vitro antibiotic susceptibility pattern but in vivo response should be observed for better results.

CONFLICT OF INTEREST

The author declares that this article's content has no conflict of interest.

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