Bacteriology of Diabetic Foot Ulcers and their Antibiotic Resistance Ability

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A thesis submitted to the Department of Mathematics and Natural Sciences in partial fulfillment of the requirements for the degree of
Bachelor of Science in Biotechnology

&

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Declaration

It is therefore declared that the thesis presented is our unique work completed while studying at BRAC University. This does not include previously published or written information by a third party, unless properly credited through complete and correct referencing. The thesis contains no material that has been approved or submitted for consideration for any other degree or certificate at a university or other institution. We have acknowledged all major sources of assistance.

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Abstract

This study is aimed to isolate the pathogenic bacteria from diabetic foot ulcers and observe their antibiotic susceptibility against antimicrobial agents. Seventy-five patients diagnosed with diabetic foot ulcers were investigated. Pus samples collected from patients were cultured in the selective media to confirm the identity of the isolated pathogenic bacteria for each patient. Further, an antibiotic sensitivity test was performed by following the Kirby- Bauer Disc Diffusion Susceptibility Test Protocol. One hundred and forty-one pathogenic bacteria were identified from the ulcer patients. *Proteus spp.* and *Klebsiella pneumoniae* are the most common pathogenic bacteria isolated, respectively. Most of the Gram-negative bacteria were resistant to Beta-lactamase group's Ampicillin. Such as *Klebsiella pneumoniae* (97.56%), *Pseudomonas aeruginosa* (95.24%), and *Escherichia coli* (81.82%). *Proteus spp*. was mostly sensitive to piperacillin-tazobactam (80%). On the other hand, Gram-positive *Staphylococcus aureus* showed mostly sensitivity towards both vancomycin and norfloxacin (79.17%).

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List of Acronyms

- **DFU-** Diabetic Foot Ulcer
- **CLSI-** Clinical Laboratory Standards Institute
- ***E.coli-*** *Escherichia coli*
- ***S. aureus -****Staphylococcus aureus*
- ***K. pneumonia-*** *Klebsiella pneumonia*
- ***Proteus spp.-*** *Proteus species*
- **TSI-** Triple Sugar Iron
- **MR-** Methyl Red
- **VP-** Vogus-Proskauer
- **MIU-** Motility Indole Urease
- **µg-** Micro-gram
- **mm-** Milligram
- **et.al-** And others

Chapter 1

Introduction

A full-thickness wound below the ankle in a person who is suffering from diabetes is known as a diabetic foot ulcer. Diabetes foot ulcer (DFU) is an open sore wound of the foot related to neuropathy, ischemia, and infection, according to the World Health Organization. Foot ulcers from diabetes (DFU) are one of the greatest challenges for diabetic patients. Patients who suffer from diabetes are estimated to have a 25% lifetime probability of developing DFU (Singh, 2005).The prevalence of diabetic foot ulcers was 1.7% and the incidence was 2.2% per year in a community-based study conducted in the northwest of the United Kingdom. Worldwide, it is anticipated that 9.1 million to 26.1 million diabetic individuals may develop foot ulcers based on the International Diabetes Federation's 2015 prevalence estimates. It is estimated that there is 26.1 million of people with diabetes and among them, 12.9 million to 49.0 million people worldwide, and 1.0 million to 3.5 million in the U. S. solely, have a back-story of foot ulceration. The number of diabetics patients who have a background of foot ulceration is greater than the amount of diabetes patients who have an active ulcer infection. People suffering from diabetes with a prior record of foot ulcers face a mortality risk that is two times higher than that of those without a foot ulcer history (Armstrong, Boulton & Sicco, 2017).

Diabetic foot ulcers are a serious condition that is the most frequent reason for no traumatic lower extremity amputations. During the course of the disease(diabetes), 14–24% of people undergo amputation of a lower extremity because of DFU(Markowitz, Gutterman, Magee & Margolis, 2006). In the US, diabetic foot ulcers (DFU) result in 80,000 amputations annually (Aumiller & Dollahite, 2015). Patients who suffer from diabetic foot ulcers are required to spend a significant amount of time in the hospital and run the risk of having a limb amputated. Patients who have diabetes have a greater than one-third chance of developing diabetic foot ulcers over the course of their lifespan (DFU). Lower limb amputations are required in 15% of DFU patients to avoid infection development (Uivaraseanu B, Bungau S, Tit DM, Fratila O, Rus M, Maghiar TA, Maghiar O, Pantis C, Vesa CM& Zaha DC, 2020). According to the International Diabetes Federation, a diabetic foot ulcer results in the removal of at least one limb (DFU) every 30 seconds somewhere in the world (Federation I. D. IDF Diabetes Atlas, 2017).

Infections can be caused by a wide variety of bacteria, Gram-negative bacteria, and Gram-positive bacteria, anaerobic bacteria (Atlaw et al., 2022). A study states anaerobic bacteria may also be present in more severe and deeper phases of the diabetic foot ulcer (F.J. Candel Gonzalez et al. 2003).*Staphylococcus aureus* is the microorganism found most frequently that causes diabetic foot ulceration.Moreover, it involves aerobic and anaerobic bacteria too. For example- *Staphylococcus aureus*, *Klebsiella species (Klebsiella pneumoniae)* , *Pseudomonas aeruginosa*, etc are the aerobic ones. Anaerobic ones are Anaerobic *Streptococci*, and *Clostridium spp. (Ogba et al.2019).* Recent research in China on adults, from age 20 to 79 years old, revealed that there were a total of 537 million patients with diabetes in 2021, and it is expected that this number would climb to 643 million by 2030 and 784 million by 2045. The study also revealed that the two main reasons for hospitalization for patients with diabetes in China are infection and ischemic diabetic foot according to (Li et al., 2022). The prevalence of diabetic foot ulcers was found to be 14.8%, 13.6%, 12%, and 4.4% in the regions of Arba Minch, Gondar, and Mekelle and Dessie, respectively, according to the research that was conducted in various parts of Ethiopia (Woldemariam et al., 2020). Infections of the diabetic foot can display both local and systemic signs of inflammation.Swelling, an increase in skin temperature, discomfort, and the development of purulent discharge are all local symptoms of inflammation. Systemic signs of inflammation include fever and metabolic disorders (Li et al., 2022).

In Bangladesh, diabetic foot ulcers are getting highly prevalent. According to a trend study, in Bangladesh, the incidence of diabetes increased from 4% in 1995–2000 to 5% in 2001–2005 to 9% in 2006–2010, respectively (Saquib et al., 2012). Further, another study showed 9.7% of the Bangladeshi adult population aged 35 or older has a prevalence of diabetes and 22.4% are prediabetes (Akter et al., 2014). As the prevalence of diabetes increases, long-term problems associated with diabetes are likely to increase. The main causes of foot and lower leg ulcers are poorly controlled diabetes or neurological impairment. A study in Bangladesh reports ulcers to develop because of a variety of factors, including insulin use (they are at high risk), being overweight, poor blood circulation, high blood sugar, a metabolic disorder caused by hyperglycemia, in this case pancreas doesn't produce enough insulin. There can be other reasons for example- feet that are sore or irritated, poor hygiene, eye disease, or kidney disease caused by diabetes. Some behavioral risk factors, including low socioeconomic position, limited access to

healthcare facilities, low educational attainment, living alone, and smoking, have also been linked to a diabetic foot ulcer (Banik PC, et al., 2020).

Despite the prevalence of diabetic foot ulcer (DFU) in Bangladesh, limited studies on its bacteriology and antibiotic resistance have been done. Besides, there is a lack of knowledge related to diabetic foot ulcers and their associated risk factors among Bangladeshi people. By conducting this research, we will be able to know the causes of diabetic foot ulcers as well as the efficacy of antibiotics in these cases. This information may help manage future cases and work as a guideline for other patients who are suffering from the same condition. For these reasons, the objective of the current research attempted to identify pathogenic bacteria that were isolated from individuals who had diabetic foot ulcers and to determine those bacteria's resistant ability to various antibiotics. This information may help manage future cases and work as a guideline for other patients who are suffering from the same condition.

Chapter 2

Methodology

2.1 Study Design:

This cross-sectional research was conducted from 22nd May 2022 to 10th November 2022 at Ibrahim Diabetic Foot Care Hospital and Shaheed Suhrawardy Medical College and Hospital. In total 75 patients' pus specimens were collected who had clinical symptoms of a bacterial foot infection/ulcer from diabetes. Samples were taken from 30 to 80 range age groups of participants and both sexes and were interviewed regarding their medical histories of diabetic foot ulcers.

2.2 Population and sample:

This study was conducted after taking written consent from study patients. Patients who met study's eligibility, which is if they showed any signs of a bacterial foot infection due to diabetes were told of its objective and participated in the study. A set of inclusion and exclusion criteria were followed while conducting this research. The inclusion study variables included patients suffer from both types of diabetes type I and type II , were at least 18 years old, as well as individuals with ulcers classified as grades 1-5 by the Wagner scale. Patients with other diabetic ulcers, those who nearly recovered with no pus cells and those 18 years old or younger were not included.

2.3 Data collection:

The questionnaire used in the study was prepared after considering different factors that are common for patients suffering from diabetic foot ulcers. A total of 15 questions are prepared and further divided into three parts (see"Appendix A"). The first part focuses on the patient's demographic characteristics, such as gender, age, location, socio-economic status, etc. This part contains 6 questions. The second part consists of questions regarding clinical characteristics like wound type, wound locality, insulin use, etc. This part contains 6 questions. The behavioral aspects of DFU patients are also included in this study which includes risky habits, foot care practices, etc. This part contains 3 questions. Patients are interviewed face-to-face as part of the data collection process. Each patient is interviewed individually for 15 minutes to get full information regarding their foot ulcer condition.

2.4 Ethical considerations:

Before starting the research, approval was obtained using both of the hospitals' ethical committees, where it would be conducted. After explaining the study to the patients, their verbal and written agreement was collected. Prior data collection, informed consent was obtained from respondents. There was an informed consent section before beginning the interview.Only individuals who consented to participate in the study self-reported and presented their information. For the convenience of the participants, the consent form was prepared in both Bengali and English for the patient's convenience (see "Appendix B"). Legal guardians of patients who were mentally unstable provided the survey information with permission. During data collection and analysis, the respondents' privacy and the confidentiality of the information were strictly ensured. The interview was completely voluntary, and the information gathered was used in an anonymous manner.

2.5 Data inspection:

The foot and ulcer were examined locally for all patients, and grading was performed using Wagner's scale method. Although several categorizations are accessible for analysis, Wagner's categorizations are a straightforward and well-approved approach for measuring and treating diabetic foot ulcers (Shah P, Inturi R, Anne D, et al.2022). All the collected data regarding patients were further analyzed. Table 1 shows Wagner's classification of diabetic foot ulcers-

Table 1: Wagner's classification of diabetic foot ulcers

Wagner's Classification	Description
0	Skin intact but bony deformities lead to "foot at risk"
1	Superficial ulcer
2	Deeper, full thickness extension
3	Deep abscess formation or osteomyelitis
4	Partial Gangrene of forefoot
5	Extensive Gangrene

2.6 Variable of the Study:

2.6.1 Dependent Variable: The presence of diabetic foot ulcers is included as a dependent variable.

2.6.2 Independent Variable: Followings are the independent variables-

- **Socio-demographic Characteristics:** These criteria include the patient's age, sex, religion, educational status, and area of residence.
- **Clinical factors:** These criteria include diabetes duration, hospital stay, type of diabetes, insulin use, and antibiotic intake.
- **Behavioral factors:** This includes behavioral risk habits like tobacco, alcohol, or other harmful substances (for example: paan, gul, etc.) consumption. Paan is usually prepared using betel leaf, betel nut, and tobacco leaf mixture. The betel nut is considered as carcinogenic by the World Health Organization (Barton et al. 2001; Parkin et al. 2005&Carpenter et al. 2005), and many studies have connected it to a increased cardiovascular disease risk and malignancies of the mouth and esophagus. Also consuming it containing tobacco makes paan very dangerous. Gul is a powdered form of tobacco that is widely available in the marketplace in Bangladesh and India.However, despite being highly addictive and carcinogenic, it is widely used for a variety of reasons and is linked to both oral cancer and potentially malignant illnesses (OPMDs) (Nathan S.T., Kumar V., Sharma S., Hariprasad R., Mehrotra R. 2018). This criteria also includes foot self-care practices, such as washing one's feet and adhering to hygiene standards, as well as whether one has a physical activity schedule (for example: yoga, walking, swimming etc.)

The classification mentioned above is done based on the study conducted in Ethiopia ((Mariam TG, Alemayehu A, Tesfaye E, Mequannt W, Temesgen K, Yetwale F, Limenih MA. 2016)

2.7 Collection of samples:

After removing the superficial secretions from the ulcer surfaces, sterile normal saline was used to clean the area. Later, a sterile cotton swab was used to firmly but gently scrape pus cells from the root of the ulcer into a tube of Amies transport media (Otta S, Debata NK, Swain B. Bacteriological profile of diabetic foot ulcers. CHRISMED J Health Res 2019;6:7-11). After that, all samples were carried carefully using an icebox, from the hospitals to the working lab. The whole collection process was done under the supervision of medical professionals.

2.8 Bacterial Culture:

All the samples were first diluted using saline and only raw,$10^{-1,}$ and 10^{-2} folds were taken for further inoculation in differential media plates (MacConkey agar, Mannitol salt agar, Cetrimide Agar, UTI Agar, KPC Agar) following the spread plate technique. The plates were then incubated at 37°C for 24-48 hours. They were later observed following colony morphology on the selective media. In Table 2, the selective media that are utilized and the colony morphology are shown.

Table 2: Colony Morphology of Specific Bacteria on Selective Media

Organism	Gram Positive/Negative	Media	Expected colony morphology
Staphylococcus aureus	Gram-positive	MSA agar	Yellow/white colonies surrounded by the yellow zone
Pseudomonas aeruginosa	Gram-negative	Cetrimide Agar	Yellow-green glow under UV ray
Klebsiella pneumoniae	Gram-negative	HiCrome KPC Agar (without added antibiotic)	Bluish-green
Escherichia coli	Gram-negative	HiCrome UTI Agar (without added antibiotic)	Blue-Purple colonies
Escherichia coli	Gram-negative	EMB Agar	Purple with a black center and green metallic sheen
Proteus spp.	Gram-negative	MacConkey Agar	Colorless colonies surrounded by the orange zone
Proteus spp.	Gram-negative	Blood Agar	Forms a thin filmy layer of concentric circles (swarming).

2.9 Biochemical tests:

Catalase test, oxidase test, triple sugar iron test, motility indole urease test, indole test, methyl red test, Voges Proskauer test, and citrate utilization test were used to further identify the selected colonies. The interpretation of biochemical tests is presented in the following Table 3:

Table 3: Biochemical Test Interpretation for Different bacteria

Organism	catalase	oxidase	Glucose ferm.	Sucrose ferm.	Lactose ferm.	Gas prod.	H2S prod.	Motility	Indole	urease	MR	VP	Citrate utilization
Staphylococcus aureus	+ve	-ve	+ve	+ve	+ve	-ve	-ve	-ve	-ve	+ve	+ve	+ve	+ve
Escherichia coli	+ve	-ve	+ve	variable	+ve	+ve	-ve	+ve	+ve	-ve	+ve	-ve	-ve
Pseudomonas aeruginosa	+ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	-ve	-ve	-ve	-ve	+ve
Klebsiella pneumoniae	+ve	-ve	-ve	-ve	-ve	+ve	-ve	-ve	-ve	+ve	-ve	+ve	+ve
Proteus spp.	-ve	+ve	+ve	-ve	-ve	+ve	+ve	+ve	+ve	+ve	+ve	-ve	-ve

2.10 Antimicrobial Susceptibility Test:

To determine the drug resistance pattern of these isolated bacteria, the Kirby-Bauer Disc Diffusion method was used to conduct an antibiotic susceptibility test. Also, the zone diameters of our selected antimicrobial agents were interpreted following the Clinical Laboratory Standards Institute (CLSI) guidelines created in 2011. The following Table 4 provides an explanation of how the Antibiotic Susceptibility test is interpreted.

Table 4: List of Antibiotics used in the Experiment

Serial no	Antibiotic	Group	Effective against	Disc code	Disc potency (µg)	Interpretative Criteria		
						Sensitive mm or more	Intermediate mm	Resistant mm or less
1	Azithromycin	Macrolide	Gram-positive and Gram-negative	AZM	15	18	14-17	13
2	Amikacin	Aminoglycoside	Gram-positive and Gram-negative	AK	30	17	15–16	14
3	Colistin	Polymyxin E	Gram-negative	CT	10	11	–	10
4	Cefepime	Cephalosporin	Gram-positive and Gram-negative	FEP	30	18	15-17	14
5	Meropenem	Carbapenem	Gram-positive and Gram-negative	MEM	10	23	20-22	19
6	Imipenem	Carbapenem	Gram-positive and Gram-negative	IMI	10	23	20-22	19
7	Piperacillin tazobactam	Penicillin and beta-lactamase inhibitor	Gram-positive and Gram-negative	PIT	100/10	21	18-20	17
8	Streptomycin	Aminoglycoside	Gram-positive and Gram-negative	S	10	15	12-14	11
9	Ampicillin	Beta-lactamase	Gram-positive and Gram-negative	AMP	10	13	11-12	10
10	Norfloxacin	Fluoroquinolone	Gram-positive and Gram-negative	NX	10	17	13-16	12
11	Vancomycin	Glycopeptide	Gram-positive	VA	30	17	15–16	14
12	Linezolid	Oxazolidinone	Gram- positive	LZ	30	21	-	20
13	Tigecycline	Glycylcycline	Gram-positive and Gram-negative	TGC	15	19	-	19

2.11 Statistical analysis:

In our study, for calculation, we have used python and panda libraries. In the fields of data science and data analysis, the Panda library is mostly used. By using the panda library, we converted our dataset into a data frame. Label encoding was done to convert those values into numeric form. Next, we used it to determine the sum and percentage.

2.12 Flowchart of the methods used in this study:

The methods that were followed in this study are shown as a flowchart in (Figure 1)

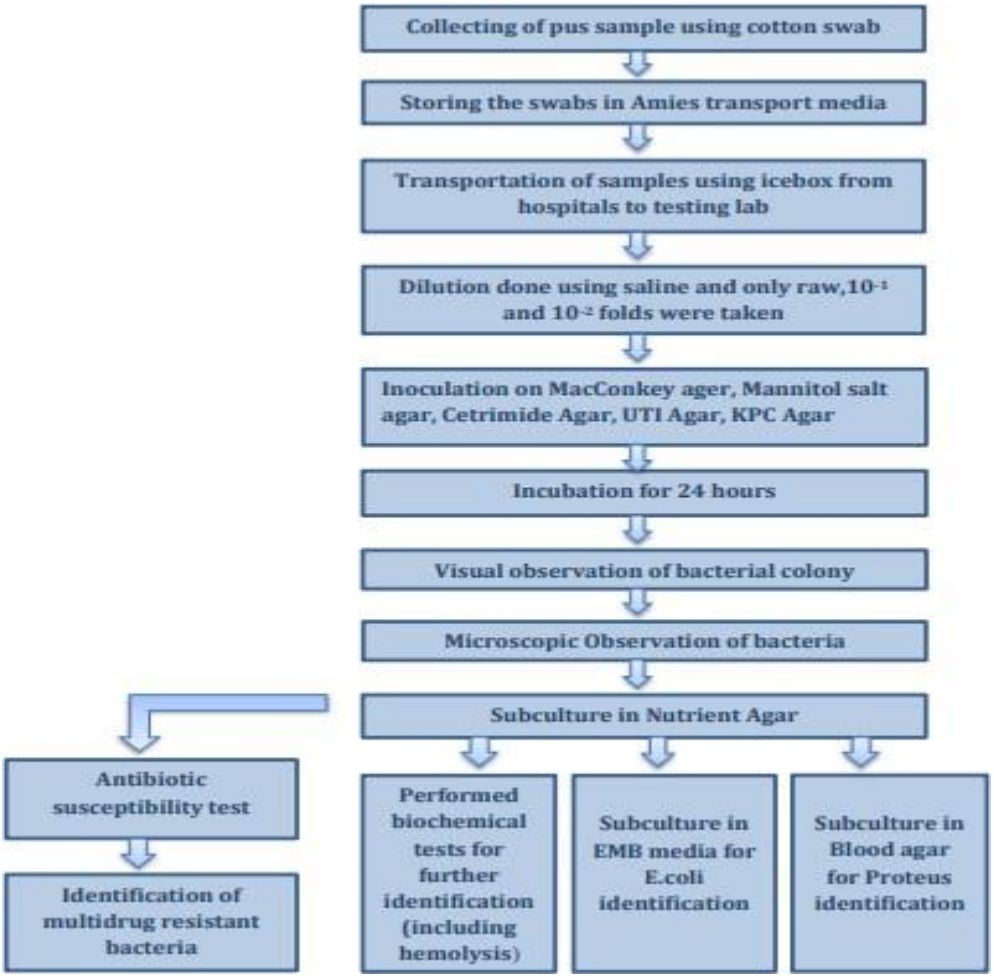

Figure 1: Flowchart detailing the methods used in this study

Chapter 3

Result

3.1 Socio-demographic characteristics:

The study comprised 75 study participants (44 from Shaheed Suhrawardy Medical College and Hospital and 31 from Ibrahim diabetic foot care hospital). There were 41(54.66%) men and 34(45.33%) women among them. The majority of participants were between the ages of 30 and 80, with a mean age count of SD ± 7.5 years. Regarding their educational status, 23(30.66%) patients have no educational background and most of them are from urban areas 53 (70.66%). The results are shown in Table 5.

3.2 Behavioral Factors:

Among the studied patients, 37(49.33%) reported having no behavioral risk habits (tobacco, alcohol, and others). Besides them, an equal percentage of people smoked and consumed betel leaf 11 (14.66%). Gross 3(4%) patients consumed alcohol in a regular manner and 4 (5.33%) of them had multiple health risk behavioral factors. A total of 46(61.33%) patients didn't maintain any physical activity schedule and only 29 (38.66%) of them had a physical activity routine. A percentage of 53(70.66%) patients did maintain proper foot self-care practices but 22 (29.33%) of them didn't do so due to ignorance and lack of knowledge regarding diabetic foot ulcers. The overall result is presented in Table 6.

3.3 Clinical characteristics:

Among the total study participants, 73(97.33%) of them had type 2 diabetes. The mean duration of a patient's diabetes is ± 12.5years. Most of the patients 59(78.66%) did intake insulin and 71(%) of them were medicated to consume antibiotics. The wound localization of each patient was observed and most of them had ulcers in the left leg 37(94.66%) following the left leg 37 (49.33%). All clinical characteristics results are reflected in Table 7.

Table 5: Socio-Demographic Characteristics

Characteristics	Categories	Frequency	Percentage
Gender	Man	41	54.66
	Woman	34	45.33
Age	30-35	2	2.66
	36-40	4	5.33
	41-45	10	13.33
	46-50	25	33.33
	51-55	7	9.33
	56-60	13	17.33
	61-65	6	8
	66-70	4	5.33
	71-75	2	2.66
	76-80	2	2.66
	Total	75	100
Number of participants at study sites	Shaheed Suhrawardy Medical College and Hospital	44	58.66
	Ibrahim diabetic foot care hospital	31	41.33
Socio-Economic Status	Employed	38	50.67
	Unemployed	37	49.33
Hospital Stay	1-6 days	19	25.33
	1 week	14	18.66
	2-3 week	16	21.33
	1 month	13	17.33
	2-3 month	13	17.33
Education	No educational background	23	30.66
	Class 1- Class 10	24	32
	SSC Pass	12	16
	HSC Pass	7	9.33
	Tertiary Education – Higher Education	9	12
Locality	Urban	53	70.66
	Rural	22	29.33

Table 6: Behavioral Factors

Characteristics	Categories	Frequency	Percentage
Behavioral risk factors	No	37	49.33
	Betel leaf (paan)	11	14.66
	Gul	4	5.33
	Tobacco	11	14.66
	Alcohol	3	4
	Multiple	4	5.33
Physical Activity	Yes	29	38.66
	No	46	61.33
Foot Self Care Practices	Maintained	53	70.66
	Not Maintained	22	29.33

Table 7: Clinical Characteristics

Characteristics	Categories	Frequency	Percentage
Type of diabetes	Type-I	2	2.66
	Type-II	73	97.33
Duration of diabetes	1-5	22	29.33
	6-10	18	24
	11-15	15	20
	16-20	12	16
	21-25	6	8
	26-30	2	2.66
Insulin use	Yes	59	78.66
	No	16	21.33
Mental stability	Normal	66	88
	Abnormal	9	12
Antibiotic Intake	Yes	71	94.66
	No	4	5.33
Wound localization	Both	5	6.6
	Right	33	44.0
	Left	37	49.33

3.4 Isolated Bacteria from Diabetic Foot Ulcer

One hundred and forty-one (141) bacterial isolates were isolated from 75 diabetic foot ulcer patients. Of these, Gram-negative bacteria are found in 117(82.97%) samples over Gram-positive bacteria in only 24 (17.02%), which solely included *Staphylococcus aureus* (Figure 2).

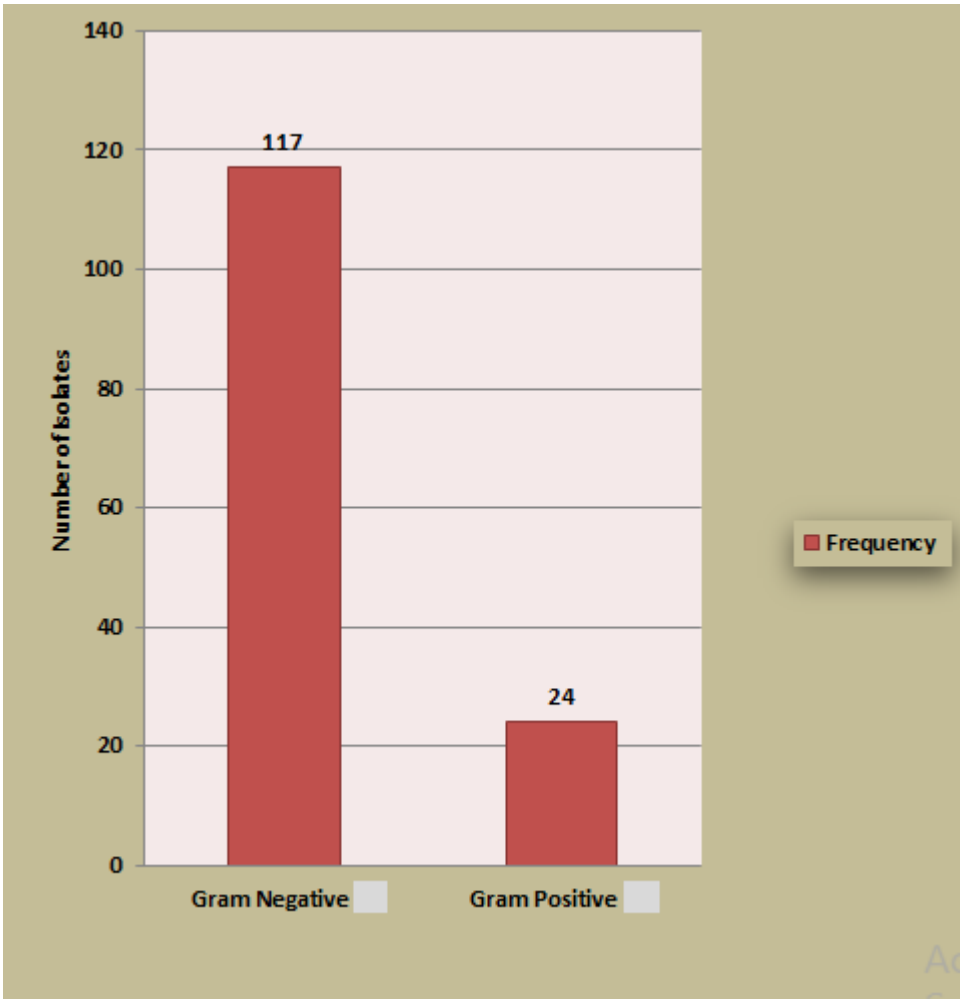

Figure 2: Isolates ratio of Gram-negative and Gram-positive bacteria

Total 18.67% of the samples showed mono-bacterial growth, 28% showed di-bacterial growth, 33.33% showed polybacterial growth, and 20% showed no growth. Among the isolated bacteria, the most predominant bacteria was, *Klebsiella pneumoniae* at 29% followed by *Proteus species* at 21.27%, *pseudomonas aeruginosa* at 17.02%, *staphylococcus aureus* at 17.02%, *Escherichia coli* at 15.60%. The number of isolates per sample is shown in Table 8 along with the prevalence of organisms.

Table 8: Identified Bacteria from Diabetic Foot Ulcer

Bacterial profiles	Name of the bacteria	Frequency	Percentage
Number of isolates per sample	Mono bacterial growth	14	18.67
	Di bacterial growth	21	28
	Poly bacterial growth	25	33.33
	No growth	15	20
Bacterial isolates	*Klebsiella pneumoniae*	41	29
	Proteus spp.	30	21.27
	Pseudomonas aeruginosa	24	17.02
	Escherichia coli	22	15.60
	Staphylococcus aureus	24	17.02

Wagner grade 2 diabetic foot ulcers had the largest number of culture-positive patients (33.33%), accompanied by Wagner grade 3 (19.85%) (Figure 3). The rate of illnesses caused by gram-negative bacteria rose as Wagner's score climbed. Wagner scale-based bacterial classification indicates that the proportion of ulcers with polymicrobial development increases as ulcer severity increases.Table 9.

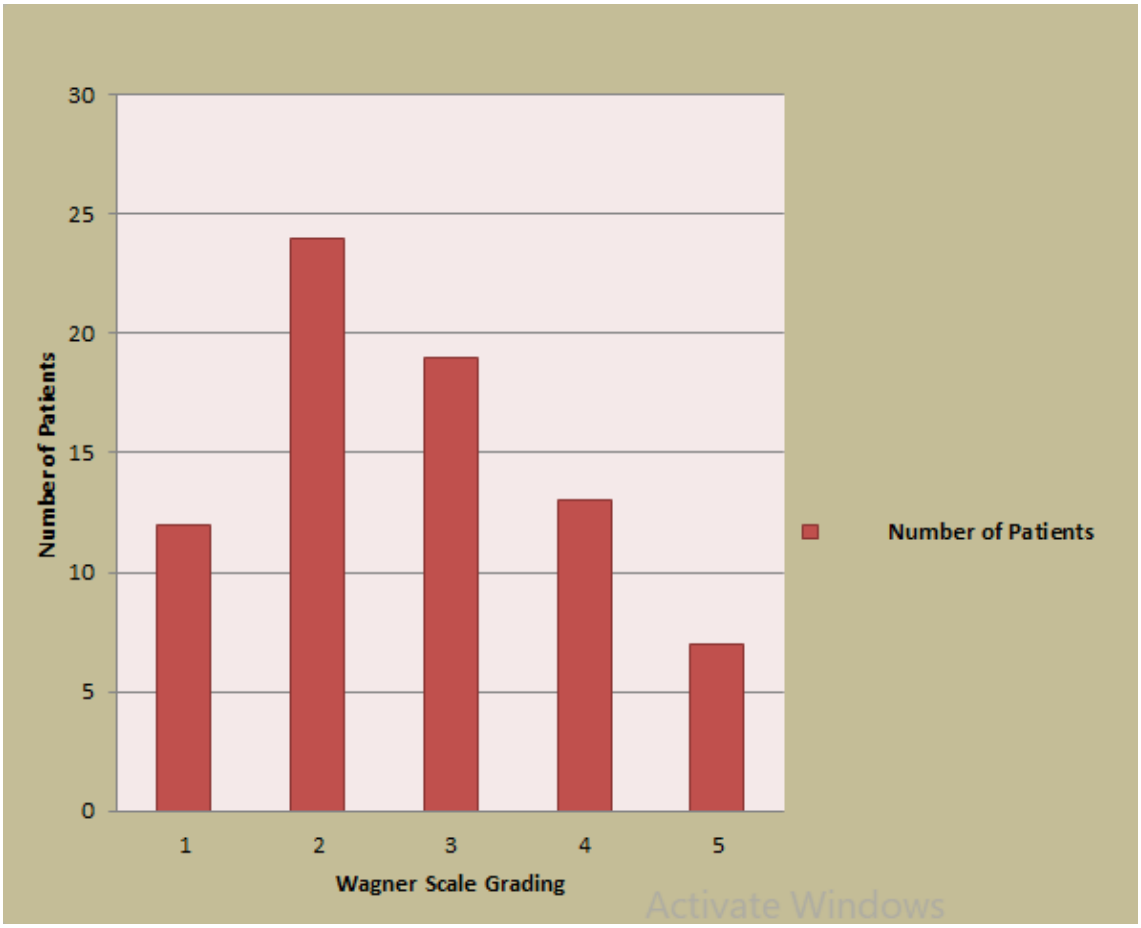

Figure 3: Wagner scale grading of the patients

Table 9: Wagner Scale based Bacterial Distribution

Isolated Bacteria	Wagner Scale Grading				
	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
S. aureus	5	10	6	2	1
Klebsiella pneumoniae	8	14	6	7	6
Proteus Spp.	6	7	7	6	4
E.coli	3	8	3	4	3
pseudomonas aeruginosa	2	8	6	4	4

3.5 Antibiotic Susceptibility Pattern of the Isolates

In this study, the Kirby-Bauer Disc Diffusion test was performed on each of the isolates. The number of isolates tested for antibiotic susceptibility was *Escherichia coli* 22 isolates, *Klebsiella pneumoniae* 41 isolates, *Pseudomonas aeruginosa* 24 isolates, *Proteus spp*.30 isolates and *Staphylococcus aureus* 24 isolates Table 10. The number of antimicrobial resistance patterns is shown in (Figure 4).

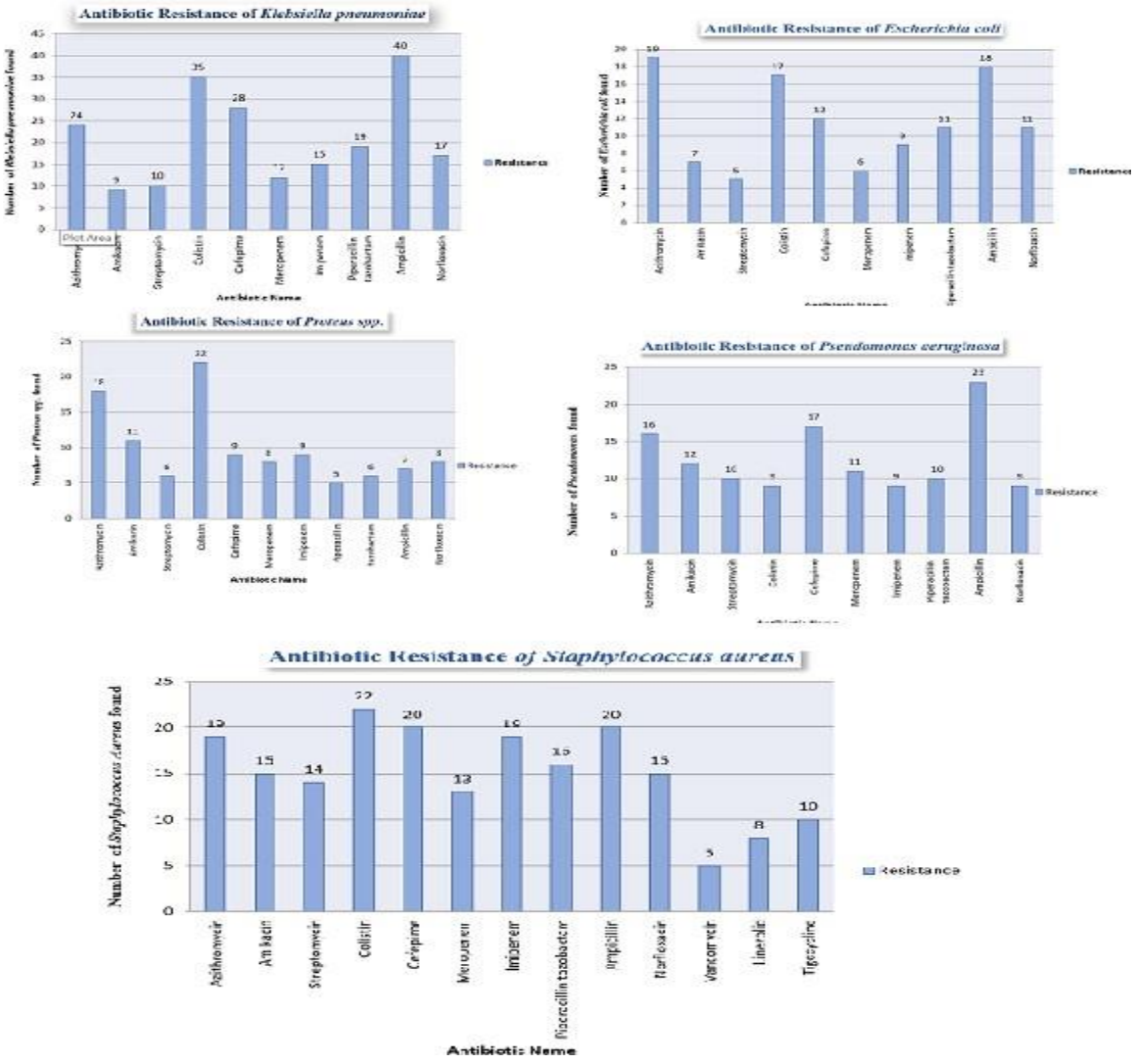

Figure 4: Antibiotic resistance pattern of the *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Proteus spp.* and *Staphylococcus aureus*

Table 10: Antibiotic Susceptibility Pattern of the Isolates

Antibiotics	Gram-Negative Bacteria												Gram-Positive Bacteria		
	Proteus Spp. (N=30)			*Klebsiella pneumonia e(N==41)*			*Pseudomonas aeruginosa (N=24)*			*Escherichia coli(N=22)*			*Staphylococcus aureus(N=24)*		
	S	I	R	S	I	R	S	I	R	S	I	R	S	I	R
Macrolide															
Azithromycin	10 (33.3)	2 (6.6)	18 (60)	17 (41.4)	0	24 (58.5)	8 (33.3)	0	16 (66.6)	3 (13.6)	0	19 (86.3)	5 (20.8)	0	19 (79.1)
Aminoglycoside															
Amikacin	19 (63.3)	0	11 (36.7)	32 (78)	0	9 (21.9)	11 (45.8)	1 (4.1)	12 (50)	15 (68.1)	0	7 (31.8)	9 (37.5)	0	15 (62.5)
Streptomycin	22 (73.3)	2 (6.6)	6 (20)	28 (68.2)	3 (7.3)	10 (24.3)	13 (54.1)	1 (4.1)	10 (41.6)	17 (77.2)	0	5 (22.7)	10 (41.6)	0	14 (58.3)
Polymyxin E															
Colistin	6 (20)	2 (6)	22 (73.3)	6 (14.6)	0	35 (85.3)	15 (62.5)	0	9 (37.5)	5 (22.7)	0	17 (77.2)	2 (8.33)	0	22 (91.6)
Cephalosporin															
Cefepime	19 (63.3)	2 (6.6)	9 (30)	13 (14.6)	0	28 (68.2)	7 (29.1)	0	17 (70.8)	8 (36.3)	2 (9)	12 (54.6)	4 (16.6)	0	20 (83.3)
Carbapenem															
Meropenem	22 (73.3)	0	8 (26.6)	28 (68.2)	1 (2.4)	12 (29.2)	13 (54.1)	0	11 (45.8)	16 (72.7)	0	6 (27.2)	11 (45.8)	0	13 (54.1)
Imipenem	20 (66.7)	1 (3.3)	9 (30)	26 (63.4)	0	15 (36.5)	14 (58.30	1 (4.7)	9 (36.9)	11 (50)	2 (9)	9 (41)	5 (20.83)	0	19 (79.1)
Penicillin and beta-lactamase inhibitor															
Piperacillin tazobactam	24 (80)	1 (3.3)	5 (16.6)	22 (53.6)	0	19 (46.3)	13 (54.1)	1 (4.7)	10 (41.6)	10 (45.4)	1 (4.5	11 (50)	8 (33.3)	0	16 (66.6)
Beta-lactamase															
Ampicillin	23 (76.6)	0	7 (23.3)	1 (2.4)	0	40 (97.5)	1 (4.7)	0	23 (95.2)	4 (18.1)	0	18 (81.8)	4 (16.6)	0	20 (83.3)
Fluoroquinolon															
Norfloxacin	17 (56.6)	2 (6.6)	11 (36.6)	21 (51.2)	3 (7.3)	17 (41.4)	15 (62.5)	0	9 (37.5)	10 (45.4)	1 (4.5)	11 (50)	8 (79.17)	1 (4.17)	15 (20.8)
Glycopeptide															
Vancomycin													19 (79.17)	0	5 (20.83)
Oxazolidinone															
Linezolid													16 (66.6)	0	8 (33.3)
Glycylcyline															
Tigecycline													14 (63.8)	0	10 (36.6)

The majority of the *Eshcherichia coli* bacteria we tested were resistant to the antibiotics azithromycin (86.36 percent), ampicillin (81.82 percent), and colistin (77.2 percent). Medium sensitivity was seen for Streptomycin (77.2%), Meropenem (72.7%), Amikacin (68.1%), and Imipenem (50%). Antibiotics like Ampicillin (97.5%) and Colistin (85.3%) show a high level of resistance against *Klebsiella pneumoniae* and a moderate level of resistance noticed in Cefepime (68.2%) and Azithromycin (58.5%). However, Amikacin (78%) shows the highest sensitivity. streptomycin (68.2%), and Imipenem (63.4%) show a moderate level of sensitivity. However, in this study, we also found *Proteus spp*. Was highly resistant to colistin (73.3%) and moderately resistant to Azithromycin (60%). Piperacillin tazobactam, Ampicillin, Streptomycin, and Meropenem show the highest sensitivity. Imipenem, cefepime, and Amikacin show moderate sensitivity for *Proteus spp*. For *pseudomonas aeruginosa*, we noticed the highest level of resistance in Ampicillin, cefepime, and Azithromycin.

Staphylococcus aureus was shown to have a high sensitivity to Vancomycin (79.17%) and a moderate sensitivity to Linezolid (66.67%) in this study. However, we also found that the isolated *staphylococcus aureus* was highly resistant to Colistin (91.67%), Cefepime (83.3%), Ampicillin (83.3%), and Azithromycin (79.1%), as well as moderately resistant to piperacillin-tazobactam (66.67%) and Amikacin (62.5%).

Chapter 4

Discussion

The majority of participants in our study reported that their ulcers usually started as tiny blisters. They thought it would be all right within a few days as it was painless and looked so small. However, due to their ignorance that little blister develops into ulcers over time. Seventy-five people who were diagnosed with diabetic foot ulcers participated in this research. Out of those, 41 (54.67%) were men and 34 (45.33%) were women. Men are more likely to develop diabetic foot ulcers than women, according to our study. Other studies have also found that men are more likely to have this problem (Gadepalli et al., 2006; Bansal et al., 2008). A high prevalence in men may be due to the greater disclosure of minor foot injuries caused by barefoot walking (Akhtar S, Latif M, Ahmed OS, Sarwar A, Alina A, and Khan MI;2022). In addition, another study conducted in India reported that 72.2% were men and 27.8% were women. This may be because men engage in more outside activities than women (Sekhar, et al. 2014). In a study in Kuwait, they reported diabetic foot ulcer was more prevalent in men than in women, and a bigger proportion of cases occurred in men at a younger age (Alhubail A, Sewify M, Messenger G, Masoetsa R, Hussain I, Nair S, et al. 2020). Contrary to our findings, a study from India reported that women had a high risk of having diabetic foot ulcers (Vibha et al., 2018). The present study findings also show, 70.67% of diabetic foot ulcer patients were from urban areas, while 29.33% of them were from rural areas. This is related to a Pakistani study that reported a higher prevalence of diabetic foot ulcers among urban diabetic patients than among rural diabetic individuals (Akhtar S, Latif M, Ahmed OS, Sarwar A, Alina A, and Khan MI;2022). In addition, the results of this study found that the age range of 46 to 50 years old has the highest prevalence of foot ulcers, which is 33.33%. In contrast, a study conducted in Pakistan revealed that diabetes patients aged 75 and older had a greater prevalence of foot ulcers (Akhtar S, Latif M, Ahmed OS, Sarwar A, Alina A, and Khan MI;2022). Another study shows most of their Patients who had diabetic foot ulcers fell into the age range of 60–79 years (Uivaraseanu B, Bungau S, Tit DM, Fratila O, Rus M, Maghiar TA, Maghiar O, Pantis C, Vesa CM, Zaha DC, 2020). The prevalence of diabetic foot ulcers among employed patients was 50.67%, in our study. On the other hand, a case-control study showed people who were employed had a 65% lower risk of developing diabetic foot ulcers than those who were unemployed (Woldemariam et al., 2020). In a study, 65.2% of respondents were currently unemployed and 67.4% claimed they were unable

to work as they're disabled. Moreover, 63.6% of respondents said they had trouble completing tasks due to diabetic foot. The study also reports walking or standing for a long time creates difficulty in their job. Also, 91.7% of men left their job and were not working because of their diabetic foot (Holloway S & Waters N; 2009)

In this study, compared to Type I, the majority of patients had Type II diabetes. 97.33 % of the people in our study had Type II diabetes, and 2.66 % had Type I diabetes. This corresponds with a study conducted in Bangladesh, where they found 45% of patients had Type II diabetes (Banik PC, Barua L & Moniruzzaman M, et al. 2020). Another study conducted in Punjab found that people with type II diabetes are more likely to develop diabetic foot ulcers than people with type I diabetes. Because Type II diabetes affects foot health more than type I diabetes (Akhtar S, Latif M, Ahmed OS, Sarwar A, Alina A, and Khan MI; 2022).

The current study identified 20% of patients with diabetes for 15 years, 16% for 20 years, and 8% for 25 years. A study conducted in south Ethiopia found 23% of the study population had diabetes for more than nine years (Deribe B, Woldemichael K& Nemera G;2014). Another study identified patients with diabetes for more than 10 years. (Uivaraseanu B, Bungau S, Tit DM, Fratila O, Rus M, Maghiar TA, Maghiar O, Pantis C, Vesa CM, Zaha DC, 2020).

In our study, we found that 78.66% of people use insulin. Another study also reports insulin users are at high risk of diabetic foot ulcers (Bhaktavatsalam M et al.2018). A study in Northwest Ethiopia found that 47% of people who use insulin have diabetic foot ulcers (Mariam TG et al. 2016).

Our study also shows that the prevalence of foot ulcers was greater among tobacco users (14.66%) than other behavioral risk factors (gul alcohol and others). Another study states tobacco users were 5.7 times more likely to develop DFU (Bhaktavatsalam M et al.2018).

In this study, patients who did proper foot care 53 (70.66%), and patients who did not take regular foot care 22(29.33%). Patients who started the foot care process early on, had less risk of major infection and some study patients reported that they delayed the foot care due to ignorance but got better results at recovering after doing so. The 29.33% of people who did not maintain any caring routine were reported to face major health issues than the ones who did. In case-

control research conducted in Eastern Indonesia, which found that patients with diabetes who routinely examined their feet had a 64% reduced risk of getting a diabetic foot ulcer than those who did not (Yusuf et al., 2016). Moreover, a cross-sectional study conducted in Iraq states that the occurrence of diabetic foot ulcers is not correlated with daily foot care (Mohammed et al., 2016). In another study, it was found that 58% of the patients didn't know much about foot care (Muhammad-Lutfi et al., 2014). According to our study, a lack of physical activity has a significant effect on diabetic foot ulcers. About 29 (38.66%) patients maintained a physical exercise schedule, while 46 (61.33%) patients did not. Non-weigh-bearing exercise has been shown to be an effective intervention to speed the healing of DFUs (Tran M& Haley M 2021).

In the present study, *Klebsiella pneumoniae* (29%) was the most common isolated pathogenic bacteria. The other predominant isolated pathogenic bacteria were accordingly *Proteus spp.* (21.27%), *Pseudomonas aeruginosa* (17.02%), *Staphylococcus aureus* (17.02%) and *Escherichia coli* (15.60%). In addition, almost 20% of ulcers had no bacterial growth. In another study *E. coli* was the predominant bacteria (Parvez et al. 2012).

The antimicrobial susceptibility pattern of the gram-positive *Staphylococcus aureus* was shown to have a high sensitivity to vancomycin (79.17%). Similarly, a study showed vancomycin was the most effective antibiotic for Gram-positive bacteria (Al Benwan et al.,2012). Further for gram-negative *Klebsiella pneumoniae* antibiotics like Ampicillin (97.5%) were most resistant. This finding is similar to the study conducted in Iran that states *Klebsiella pneumoniae* showed highest resistance to ampicillin (96%) (Kashefieh et al.,2021).

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Appendix

Appendix A

Questionnaire

Diabetic Foot Ulcer Study Questionnaire

Patient name: _

Hospital name: _

1. **Sex : □ Man □ Woman □ Others**
2. **Age :**
3. **Living location:**
4. **Socio economic status :**
    - **Job :**

5. **Duration of diabetes:**
6. **Duration of hospital stay:**
7. **Type of diabetes:**
8. **Insulin use:**
9. **Behavioral risk factors: □Tobacco □ Alcohol □Others**
10. **Physical Activity:**
11. **Localization of wound:**
12. **Type of wound:**
    - **Grade 1:**
    - **Grade 2:**
    - **Grade 3:**
    - **Grade 4:**
    - **Grade 5:**
13. **Foot hygiene condition:**
14. **Mental stability:**
15. **Additional notes:**

Interviewed By- _

Appendix B

Consent Form

Greetings from BRAC University. We are working to find out the bacteriology of diabetic foot ulcer and their antibiotic resistance. We are communicating with a request to cooperate with us by providing us information regarding your symptoms, treatment, and current conditions concerning 'Diabetic Foot Ulcer (DFU)'. This will take about 15 minutes. Your response will help us immensely in providing a revelation of how the diabetic foot ulcer is caused and how efficient antibiotics are in this case, which may help manage future cases and to design guidelines for the other patients suffering from the same condition.

Your participation in the survey is voluntary. Your information from this survey will be kept confidential. This is non-profitable research work. If you agree to participate, please sign this consent form with your proper information and proceed to answer the questions kindly. Thank you.

For further questions regarding this survey please contact….01715299027..

01748679588

Name:

Age:

Contact information:

Signature: _

সম্মতি পত্র

ব্র্যাক বিশ্ববিদ্যালয়ের (BRAC University) পক্ষ থেয়ক আপনায়ক শুয়েচ্ছা। আমরা ডাে◌ায়িটিস থরাগীয়দ্র পায়ের ক্ষত এি◌ং এই অি◌স্থা◌ে এবিি◌ায়োটি◌ক গ্রহণ কতি◌◌া কার্করী তার উপর অনসন্ধা◌ান করি◌। এই অসুস্থতার সময়◌ে আপন◌ার লক্ষণ, গৃহী◌ত বি◌◌কৎসা, এি◌◌ং ি◌তমান অি◌স্থা জাবনয়◌ে এই জবরয়প অংশগ্রহণ
করয়ত আপন◌ার সহয়র্াবগতা কামনা করি◌। এই জবরপ পূরয়ণ সয়ি◌◌্চ ১৫ বমবনি◌ লাগয়ি◌। আপন◌ার থদ্◌◌ো
মূলযি◌◌ান তেয ডা◌ে◌◌ায়িটি◌স জবনত পায়ে◌র ক্ষতর উৎস ত্রিং এবিি◌◌ায়োটি◌ক এই রী তা থক্ষয়◌ে কতি◌◌ু কু কার্করনর্◌ারয়ণ সহা◌েতা করয়ি◌,র্া ে◌বিষ◌্যয়ত একই অি◌স্থা◌ে আক্রান্ত অনয◌ানয থরাগী◌য়দ্র জনয বনয়দ্◌শকা পবরকল্পনা করয়ত সাহার্য করয়ত পায়র।

এই জবরয়প আপনার অংশগ্রহণ থেচ্ছাপ্রসূত।জবরয়প আপনার থদ্◌◌ো র্া◌িতী◌ে তয়েযর থগাপনী◌েতা রক্ষা করা হয়ি◌।এটি একটি অলা◌েজনক গয়িষ্ণা কাজ। আপবন র্বদ্ এই জবরয়প অংশগ্রহণ করয়ত সম্মত হন, অনুগ্রহ কয়র আপন◌ার সটি◌ক তেয সহ এই সম্মবত ফয়ম ে◌◌াক্ষর করুন ত্রিং দ্◌ে◌◌া কয়র প্রয়নাত্তয়র অংশগ্রহণ করুন। র্নয◌ি◌◌াদ্।

জবরপ সংক্রান্ত থকান প্রন থেয়ক ে◌◌াকয়ল ...০১৭১৫২৯৯০২৭ .....নম্বয়র থর্াগায়র্াগ করুন।

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নাম:

বয়স :

যQাগাযQাযগর ি◌থ্য:

স্বাক্ষর: _