Chapter-1: Introduction

1.1 Introduction

Diabetes mellitus (DM) is a metabolic disorder, affecting a large segment of population. It is one of the chronic noncommunicable diseases (CNCDs) which is a leading global health problem (Ahmed *et al.,* 2006). In the last few decades, especially in the developing world, diabetes is the fourth leading cause of deaths (Alavi *et al.,* 2007). According to epidemiological studies, the number of patients with diabetes mellitus (DM) increased from about 30 million cases in 1985, 177 million in 2000, 285 million in 2010, and it is estimated that if the situation continues, more than 360 million people by 2030 will have DM.

Hereditary, ecological, and metabolic risk factors contribute to the development of type 2 diabetes mellitus (T2DM) and are interrelated. Higher risk of diabetes with a family history (FH) of diabetes mellitus, age, obesity, and physical inactivity has been identified. Influence of dietary habits and lifestyle are critical and are responsible for higher occurrence and prevalence of obesity and diabetes in the urban population (Arun *et al.,* 2016; Boulton *et al.,* 2005). The prevalence of diabetes in Bangladesh is increasing rapidly, leading to chronic complications of diabetes. It is also a known risk factor for retinopathy which may develop into blindness, vascular brain diseases, nephropathy, and limb amputations etc.

Diabetic foot is one of the feared complications associated with diabetes and affects quality of life in respective patients in all ages and races (Citron *et al.,* 2007). The annual incidence of leg and foot ulcers is 2, 6.5 and 33 times more common than diabetic coronary disease, stroke and renal failure respectively. About 15% of diabetic patients develop a foot ulcer during their lifetime and 20% suffer from some type of foot infection in their lifetime (Dang *et al.,* 2003; Ellen *et al.,* 2017; Gary *et al.,* 1997). This leads to a devastating sequela causes significant mortality and morbidity and poses a substantial amount of financial burden on our health care (Thaker *et al.,* 2013). Rate of amputation of a limb is estimated to be forty times greater in infected non healing ulcer in diabetics than the patients of trauma (Herbert *et al.,* 2017). Peripheral sensory and motor neuropathy leading to deformities, macro and

microangiopathy leading to ischemia, and infection are the major etiologies of diabetic foot (Hartemann *et al.,* 2004).

In addition foot complications now account for the most frequent reason for hospitalization in diabetic patients (Jagadeesha *et al.,* 2019). Whenever an infection has developed in Diabetic foot ulcer patients, it is a challenge for physicians to treat it because of impaired microvascular circulation to the lower limb, which limits the access of phagocytic cells and antibiotics to infected sites (Yahya *et al.,* 2016). Various microorganisms like Gram-negative bacteria, Gram-positive bacteria and few fungal species are reported as the common microbes present in diabetic foot infections and in some patients one or more species of organisms proliferate in the wound, which may lead to tissue damage, host response accompanied by inflammation, that is, clinical infection (Joseph *et al.,* 2013; khan, 2006).

Chronic subclinical inflammation (CSI) reportedly has a significant association with the development of acute diabetic foot syndrome (Lipsky *et al.,* 2004). Association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers is increasing gradually resulting challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation. However, appropriate antibiotics is mandatory to avoid the risk of severity in foot infections of diabetic patients. But the presence of drug-resistant bacteria makes the antibiotic therapy more difficult (Turhan *et al.,* 2013).

The objective of the study is to identify risk factors, analysis of co-morbidities and complications of DM and therefore to isolate microorganisms in patients with infected diabetic foot ulcer using culture-based methods and antibiotic sensitivity pattern of microorganisms so that it will be helpful in prescribing appropriate antibiotics, reducing hospital stay, preventing major surgical interventions and thereby saving resources.

1.2 Objectives

1.2.1 General objective

Identification and isolation of microorganisms involve in diabetic foot ulcer and to find antibiotic sensitivity pattern of isolated microorganisms to reduce the diabetic foot complications.

1.2.2 Specific objectives

1. To quantify the risk factors of patients developing diabetes.
2. To analyze comorbidities and complications of diabetes mellitus.
3. To isolate microorganisms from diabetic foot ulcer involve in diabetic foot infections.
4. To find out Antibiotic sensitivity pattern of isolated microorganisms from diabetic foot ulcer.
5. To determine the appropriate antibiotic to treat diabetic foot ulcer.

Chapter-2: Review of Literature

The increase in prevalence of diabetes mellitus (DM) is being associated with many complications among diabetic patients. Foot complications are a leading cause of mortality in developing countries.

2.1. Gender based prevalence of diabetic foot

Previous reports indicated that the prevalence of diabetic foot (DF) among males and females was 58.0% and 52.9%, respectively, without significant difference between both sexes. Eighteen percent of study population reported history of foot ulcer. Almost 53.6% patients had good foot care knowledge. Gender, duration of DM, marital status and age had no significant association with knowledge. Males were more adherent to foot drying by 65.2%, while females are applying more attention to softening of skin by 72.3%. There were no significant differences between males and females regarding foot inspection, nail care, adherence to medication and shoes check (Yahya *et al.*, 2016).

2.2. Diabetes mellitus as a public health concern

2.2.1. Epidemiology of diabetes mellitus

Diabetes mellitus (DM) is considered as one of the most challenging public health concerns, as globally 422 million adults were living with diabetes in 2014, compared to 108 million in 1980. The global prevalence of diabetes has nearly doubled since 1980, rising from 4.7% to 8.5% in the adult population. One of the major complications associated with DM is the diabetic foot (DF) disease. This complication almost affects 50% of patients and accounts for nearly 80% of all nontraumatic amputations of the lower limb. The disease represents nearly 35% of all hospital admissions in diabetic specialized clinics. DF complication is the major cause of a significant loss of quality and years of life of diabetic patients. In term of cost, it represents 12-15% of the overall cost associated with diabetes and up to 40% in developing countries (Smith *et al.*, 2002).

Foot ulcers are a significant complication of diabetes which are the most common cause of nontraumatic lower extremity amputations in the industrialized world. The risk of lower extremity amputation is 15 to 46 times higher in diabetics than in

persons who do not have diabetes mellitus. Furthermore, foot complications are the most frequent reason for hospitalization in patients with diabetes. Careful inspection of the diabetic foot on a regular basis is one of the easiest, least expensive and most effective measures for preventing foot complications.

Appropriate care of the diabetic foot requires recognition of the most common risk factors for limb loss. Many of these risk factors can be identified based on specific aspects of the history and a brief but systematic examination of the foot.

Foot infections are the most common complications of diabetic foot and plays a main role in the development of moist gangrene. *Pseudomonas* spp., *Enterococcus* spp. & Proteus spp. carry a special role and are responsible for continuing and extensive tissue destruction with the poor blood circulation of the foot.

A high frequency of anaerobic infection has also been reported. Seven patients with diabetes also can have a combined infection involving bone and soft tissue called fetid foot. This extensive soft tissue and bone infection causes a foul exudate, is chronic, and usually requires extensive surgical debridement and/or amputation (Smith *et al.*, 2002; Seyed *et al.*, 2007).

2.2.2. Burden of Diabetes mellitus in community

In general, people with diabetes have infections that are more severe and take longer to cure than equivalent infections in other people. The infection leads to the early development of complication even after a trivial trauma, the disease progresses and becomes refractory to antibacterial therapy. It is essential to assess the magnitude of bacterial infection of the lesions to avoid further complications and save the diabetic foot. Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy and to avoid further complications.

However, these infections are difficult to treat because these patients have impaired microvascular circulation, which limits the access of phagocytic cells to the infected area and results in a poor concentration of antibiotics in the infected tissues. For this reason, cellulitis is the most easily treatable and reversible form of foot infections in patients with diabetes. Deep skin and soft tissue infections also usually are curable, but they can be life threatening and result in substantial long term morbidity.

In terms of the infecting microorganisms and the likelihood of successful treatment

with antimicrobial therapy, acute osteomyelitis in people with diabetes is essentially the same as in those without diabetes. Chronic osteomyelitis in patients with diabetes mellitus is the most difficult infection to cure. Adequate surgical debridement, in addition to antimicrobial therapy, is necessary to cure chronic osteomyelitis.

To study the relative frequency of bacterial isolates cultured from diabetic foot infections and assess them in vitro sensitivity to the commonly used antibacterial agents, a prospective microbiological study was carried out and results are presented here (Seyed *et al.,* 2007).

2.2.3. Burden of Diabetes mellitus in a global scale

2.2.3.1. Burden of Diabetes mellitus in gulf

Type 2 diabetes mellitus is one of the major chronic disease burdens with a prevalence of 422 million patients worldwide. Type 2 diabetes is expected to be the seventh most common cause of death in the world by 2030, primarily due to its rapid rise in middle-income and low-income countries. In addition, type 2 diabetes is a leading cause of severe morbidities and disabilities (blindness, chronic renal impairment, cardiovascular events, and lower limb amputation). Within the Gulf region, the prevalence of type 2 diabetes and associated risk factors and comorbidities is one of the highest in the world (Ellen *et al.,* 2017).

In the United Arab Emirates (UAE), specifically, the prevalence of prediabetes and type 2 diabetes were reported to be 30% and 23%, respectively,6 with 6.6%–14.6% of UAE residents remaining undiagnosed. A screening among Emirati children and adolescents showed that 5.4% are already in the prediabetes state,9 indicating that type 2 diabetes constitutes a considerable future challenge in this country. In addition, UAE has high rates of overweight, Clinical profiles, comorbidities and complications of type 2 diabetes mellitus in patients from United Arab Emirates School of Community Health.

In the United Arab Emirates (UAE), the prevalence for type 2 diabetes is 23%, with hypertension, obesity, and dyslipidemia as common type 2 diabetes comorbidities. What are the new findings? Few studies have been reported on Arab population regarding type 2 diabetes and its complication, and we have found that more than 80% of patients with type 2 diabetes in UAE have one or more complication with

retinopathy (13.26%), being the most frequent single complication (Seema *et al.*, 2009).

2.2.3.2. Burden of Diabetes mellitus in Asia

Type 2 diabetes, obesity and hyperlipidemia have been traditionally considered as diseases of affluence. A wealth of data indicates that Asian Indian people abdominal obesity and insulin resistance and develop glucose intolerance more often. The prevalence of diabetes is higher in migrant Asian Indian people as compared to other ethnic groups. Some of the studies done on native Indian people also show high prevalence of diabetes in urban areas. Contribution of dietary practices and lifestyle factors are crucial, making incidence and prevalence of obesity and diabetes mellitus significantly more in the urban population. Recently, considerable concern has been caused by the increasing prevalence of diabetes in India, particularly in the urban population.

High prevalence of malnutrition in people belonging to low socio-economic strata in developing countries led to the assumption that obesity and diabetes will not be a crucial problem in them. Whereas a rural population usually has low risk of development of diabetes and obesity in India,7 their migration to metropolitan cities exposes them to several adverse lifestyle and environmental influences (Shailesh *et al.*, 2012).

In cities they usually settle down in urban slums, and take to daily wage jobs. Several lifestyle alterations result from this transition: changes from their traditional penurious eating habits; exposure to severe stress; decreased physical activity; and increase in smoking, tobacco chewing and alcohol intake. Unfortunately, this population has not been researched in detail. A few studies from the developed countries indicate that the prevalence of established risk factors including obesity and diabetes mellitus are higher among men and women with low level of education as a measure of socioeconomic status. A recent study from the UK records that type 2 diabetes is inversely related to socio-economic strata.

In the study, the prevalence of diabetes in the least deprived quintile was 13.4 per thousand persons (95% CI 11.44– 15.36), compared to 17.22 (95% CI 13.84–17.11) in the most deprived. However, in developing countries, poverty and scarcity of food

is greater, awareness of diseases non-existent, and it appears that these diseases may be equally prevalent in poor people. In one of the largest studies to date, obesity in 6.4% of the boys and 8.7% of the girls, in 2411 subjects from 535 families living in the shanty towns of Sa ˜o Paulo, Brazil.13 In this population, there was 30% prevalence of malnutrition, and 78–90% prevalence of stunted growth in children. Even in this population, high prevalence of overweight (16.7%) and obesity (14.1%) was noted in adults (A. Misra *et al.,* 2001).

Moreover, in 9% of the families, malnutrition in children and obesity in adults co-existed. To study the lifestyle, anthropometric and metabolic attributes of such a population of low socio-economic strata, we attempted a cross-sectional prevalence survey of obesity, diabetes mellitus, hyperlipidaemia and related lifestyle factors in an urban slum in New Delhi (Delhi Urban Slum Survey), the largest metropolitan city in northern India (Boulton *et al.,* 2005).

2.2.3.3. Burden of Diabetes mellitus in Latin America

The occurrence of T2DM in the Brazilian population has increased considerably in recent years, and this is currently one of the most prevalent chronic diseases in the country. This increase is probably related to habits of the modern world, such as the consumption of high-energy diets and sedentary lifestyle, as well as increased life expectancy, development of obesity and difficult access to health services. In addition, there are genetic factors that favor the disease, which makes some people more susceptible to it. Diabetes is a pathology that stands out for the potential of developing long-term complications.

2.3. Foot ulcers as a complication of diabetes mellitus: Bangladesh perspective

2.3.1. Prevalence of foot ulcers in Bangladesh

Foot ulceration and infections are perhaps the most frequent and serious complication of diabetes mellitus (DM). The annual incidence of leg and foot ulcers is 2, 6.5 and 33 times more common than diabetic coronary disease, stroke and renal failure respectively. About 15% of diabetic patients develop a foot ulcer during their lifetime

and 20% suffer from some type of foot infection in their lifetime. Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders (BIRDEM), a central referral hospital in Dhaka city, provides basic diabetes care to a large number of diabetic population.

The total number of registered patients in BIRDEM is >3,20,000 and daily turnover is around 2500. A retrospective cohort study from 1980 to1995 among patients in BIRDEM showed a 2.8% prevalence of diabetic foot ulcer. Many studies have reported on the bacteriology of diabetic foot infection over the past 25 years, but the results have varied and have often been contradictory. A number of studies have found that *Staphylococcus* aureus is the main causative pathogen. But recent investigations reported a predominance of Gram negative aerobes. Several studies have confirmed that chronic lesions or infections receiving prior antibiotic treatment are usually polymicrobial.

The study detect the bacteria responsible for diabetic foot infections among patients attending the out and in-patient departments of BIRDEM hospital. Most of our patients had grade 3 ulcers. Our study shows that in chronic, complex and previously treated wounds, infections are generally polymicrobial with mixed Gram positive and Gram negative organisms. We found Gram negative aerobic bacteria as the most frequently isolated organism though previous studies had shown Gram positive aerobes as the predominant organisms in DFI.9,14,18,19 Thus the major infective organisms in diabetic foot ulcer in our patients appear to be different. The ratio of Gram positive to Gram negative was 1:4 (Samir *et al.*, 2009; Ahmed *et al.*, 2006).

2.3.2. Gender based difference in ulcerative complications

The differences in the age-sex composition and ulcer grades between the study population and those of earlier studies might be the reason for these differences. However, our results are in tune with other studies done in India which also showed that Gram negative bacteria were the most predominant organisms in DFI.10,11 The role of anaerobic organisms in DFI could not be determined as no attempt was made in this study to isolate the anaerobes. High levels of resistance to ciprofloxacin, cotrimoxazole, amikacin, gentamicin and cephalosporins were found in all isolated organisms. Only Imipenem was the most effective agent against all Gram negative

organisms.

High rates of antibiotic resistance observed in a study may be due to the widespread use of broad spectrum antibiotics in the tertiary care hospital leading to survival advantage of resistant pathogens. About 31.5% Gram negative bacteria were ESBL producers and 43.8% of S. aureus were methicillin resistant. The increasing prevalence of ESBL producing organisms and MRSA is disconcerting, because infection with these organisms limits the choice of antibiotic treatment and may lead to a worse outcome (Dang *et al.,* 2003).

2.3.3. Sequels of ulcerative complications

Foot ulcers are much feared complications of diabetes, with recent studies suggesting that lifetime risk of developing a foot ulcer in diabetic patient may be as high as 25%. Fifteen per cent of people with diabetes will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer.

Infection is most often a consequence of foot ulceration, which typically follows trauma to a neuropathic foot. Severe infections in the foot may lead to leg amputations. In addition, foot complications now account for the most frequent reason for hospitalization in diabetic patients. Gram-negative bacteria, Gram-positive bacteria and few fungal species are reported as the common microbes present in diabetic foot infections.

Diabetic foot infections are sores on the feet that occur in 15% of diabetic patients some time during their lifetime. The risk of lower-extremity amputation is increased 8-fold in these patients once an ulcer develops. Foot disorders such as ulceration, infection, and gangrene are the leading causes of hospitalization in patients with diabetes mellitus.

Foot ulcers are a significant complication of diabetes mellitus and often precede lower-extremity amputation. The most frequent underlying etiologies are neuropathy, trauma, deformity, high plantar pressures, and peripheral arterial disease. Thorough and systematic evaluation and categorization of foot ulcers help to guide appropriate treatment.

Foot ulceration is common, affecting up to 25% of patients with diabetes during their lifetime. Over 85% of lower limb amputations are preceded by foot ulcers and

diabetes remains a major cause of non-traumatic amputation across the world with rates being as much as 15 times higher than in the non-diabetic population (Angger; 2018).

2.4. Organisms associated with diabetic foot ulcers

2.4.1. Overview on ulcer causing organisms

Multidrug resistant Gram-negative bacteria (MDRGNB) are a major therapeutic challenge both in hospital and community settings. The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation. Hence, usage of appropriate antibiotics is needed to avoid the risk of severity in foot infections of diabetic patients. But the presence of drug-resistant bacteria makes the antibiotic therapy more difficult.

Anaerobic bacteria are almost always isolated with aerobes from diabetic foot infections. Aerobic gram-positive cocci are the predominant microorganisms that colonize and acutely infect breaks in the skin. S. aureus and the beta-hemolytic streptococci (groups A, C, and G, but especially group B): Enterococci, Enterobacteriaceae, obligate anaerobes, *Pseudomonas* aeruginosa, non-fermentative gram-negative rods. Antibiotic-resistant organisms: (e.g., MRSA or vancomycin resistant enterococci) (Mojtaba *et al.,* 2015).

Coagulase negative staphylococci and Corynebacterium species (diphtheroids). Sometimes, initial management comprises: multidrug resistant Gram-negative bacteria (MDRGNB) are a major therapeutic challenge both in hospital and community settings. The pathogenic role of each isolate in a polymicrobial infection is often unclear. The high prevalence of anaerobic bacteria in the foot ulcers of diabetic patients was first documented by Louie and colleagues in 1976.

The detection of neuropathy before it gets severe is the best method to prevent diabetic foot infections (Shakil *et al.,* 2008; Yoga *et al.,* 2006).

2.4.2. Methods of collecting samples from ulcer wounds

The Foot ulcer samples were collected from patients who had Type 2 diabetes and subjected to microbiological analyses. Sample collection (pus, wound exudates) had

been undertaken in medical wards, after the wounds are washed vigorously with normal saline solution. Discharge from margins and edges of ulcer was collected with help of two sterile swabs, one for gram stain and one for culture before antiseptic dressing was applied. Then swabs were immediately transported to the laboratory for culture. A total of 530 samples were collected from 530 patients. Out of 530, 410 (77.4%) were males and 120 (22.6%) were females. The female: male ratio in this study was 1: 3.41. The age range was 40 90. Then using various differential and selective media, the samples were cultured aerobically and the aerobic bacteria were isolated (Seyed *et al.,* 2007).

2.5. Sensitivity of ulcer causing organisms

2.5.1. Overview on concepts related to sensitivity testing

Based on the results from sensitivity testing, the isolated bacteria showed 65% resistance to used antibiotics. This was a higher resistance compared to similar work of Hartemann *et al.* (2004) which they yielded 18% multidrug resistance.20 S. aureus showed high resistance to Cloxacillin (91%), Amoxycillin (91%), Ceftazidime (72%), Vancomycin (63%) and Clindamycin (54%), which the resistance was higher compared to study of Pathare et al, as they reported 40% resistance in this organism to similar antibiotics.

S. aureus showed good sensitivity to Ciprofloxacin as the similar results were reported previously by Tahawy.17 All the gram negative isolates showed 100% resistance to used antibiotics except for Proteus mirabilis which the resistance rate was 50%. Besides isolates of *Klebsiella*, Proteus vulgaris and *Pseudomonas* aeruginosa were fully sensitive to Ciprofloxacin (Hartemann *et al.,* 2004).

It seems that the status of multidrug resistance among the majority of isolates in study, was not associated with patient characteristics (age, sex, type and complications of diabetes), wound duration or wound type (neuropathic or ischaemic), while a history of previous hospitalization for the same wound was very important in emergence of resistant organisms. (Jagadeesha *et al.,* 2019).

Winkler *et al.* (1972) used a modified gram staining technique to show that 13 of 15 specimens showed predominately gram positive cocci and rod shaped bacteria uniformly dispersed throughout the periapical lesions stained. They attributed the

development of the periapical granulomas to a bacterial invasion of apical tissue.
A review of many of these early studies, however, leaves room for doubt as to the control of contamination during tissue retrieval as well as concern about potential anaerobic organisms that may have been lost due to the lack of anaerobic isolation and culturing techniques.

2.5.2. Concerns related to sensitivity testing

With the greater recognition of the importance of maintaining anaerobic conditions during specimen retrieval, more recent studies have yielded more information on the possible microbes associated with periapical pathosis. Among the more common isolates in these studies were the black pigmented Bactericides, *Porphyromonas spp*., *Prevotella spp.,* and facultative anaerobes like *Streptococci spp*., *Actinomyces spp., Lactobacillus spp.,* and *Peptostreptococci spp*. The results from these studies are some- what varied aud the possibility for specimen contamination cannot be ruled out. lwu *et al.* attempted to clarify the conflicting information in the literature.
The design for their study included the first real attempt at washing periapical tissue removed during endodontic surgery in an effort to remove possible contamination from adjacent tissues. Of 16 periapical granulomas studied, 14 (88%) yielded positive culture after washing and grinding. A mixed population of strict and facultative anaerobes was isolated in 10 specimens. Two lesions yielded strict anaerobes and two others a pure culture of facultative anaerobes. The organisms isolated differed markedly in both kind and number from previous studies. In a corroborative study using 58 periapical lesions of varying kinds Wayman *et al.* also reported a mixed population of aerobes, facultative anaerobic, and obligate anaerobes. The organisms identified differed somewhat from lwu's results, and, more importantly, no attempt was made to wash the tissue specimens following removal from the surgical field. In two related studies involving the last 5 mm of root and attached soft tissue (Mathangi1 *et al.*, 2013).

2.5.3. Colonization of organisms

Chronic wounds can be colonized on the surface by a wide range of organisms. Several studies have shown different bacterial agents isolated from patients in different geographical areas in Iran. The inconsistency in reports might be attributed to the varying research methods and populations. If bacterial infection is mild, it is usually monobacterial and if severe infection is present, it is polymicrobial. The antibiotic sensitivity patterns also show variations in diverse geographical regions. Multidrug resistant (MDR) bacteria, methicillin resistant S. aureus (MRSA), and extended-spectrum β-lactamase (ESBL) producing Gram-negative bacteria and their associated complications have created a big health concern among the medical and clinical practitioners.

In recent decade, high rates of MDR bacteria, MRSA, and ESBL positive strains have been observed in many hospitalized diabetic foot patients (DFP). Such conditions make the treatment more demanding and many even menacing to the respective hospitalized patients' lives. Therefore, early diagnosis of lesions and prompt initiation of appropriate antimicrobial therapy are essential for controlling the infection and preventing complication and improving the quality of life. Antibiotic sensitivity test is a requirement for the management of infections which can help to make better therapeutic choices. Hence, this study was designed to evaluate the prevalence of microorganisms in infected diabetic foot cases and their sensitivity patterns in public hospital, in Fars, Shiraz, Southern Iran (Varma *et al.*, 1986).

Chapater-3: Materials and Methods

Following approval by the Ethical and Research Committee of Chattogram Veterinary and Animal Sciences University (CVASU) an observational study was carried out to explore the risk factors of diabetic patients, identification of microorganisms from diabetic foot ulcer and antibiotic sensitivity pattern. Informed consent was obtained from diabetic patients who were included for the study. The findings on grading of foot ulcer were compared based on sex, occupation, duration of diabetes, risk factors, comorbidities etc.

3.1. Study design: This was a descriptive type of cross-sectional study.

3.2. Study period

This was a 06 (six) months study commencing from 1st January, 2020 to June, 2020. For the purpose of the study the total study period was divided into different parts based on the tasks of the study including topic selection, ethical approval, questionnaire development, data collection, data analysis, manuscript writing etc as detailed in appendix A.

3.3. Place of the study

1. Chattogram Diabetic general hospital, Chattogram

Chattogram Diabetic Association (CDA) is a non-profit voluntary socio-medical organization branch of Diabetic Association of Bangladesh, Dhaka is registered with the Ministry of Social Welfare under the Society's registration act, 1860. Though Chattogram Diabetic Association was founded in 1975, by the guideline of National Professor Dr. Muhammad Ibrahim and taking help from a group of dedicated social workers, physicians and civil servants of chattogram city. It has become the second largest diabetic hospital in Bangladesh.

3.4. Reference population: All diabetic patients with foot ulcer hailing from Chattogram district.

3.5. Source population: Diabetic patients those who developed foot ulcer admitted in Chattogram Diabetic General Hospital, Chattogram.

3.6. Sample size:

This study included 106 participants from Chattogram Diabetic General Hospital, Chattogram.

3.7. Sampling technique: Non-probability type purposive sampling was done for the study.

3.8. Selection criteria

3.8.1 Inclusion criteria

1. Diabetic patients with foot ulcer admitted in Chattogram Diabetic General Hospital.
2. Just after admission. (9 am to 2 pm).

3.8.2 Exclusion criteria

1. Diabetic patients with foot ulcer admitted in Chattogram Diabetic General Hospital before 9 am and after 2 pm.

2. Patients not willing to give written consent.

3.9. Research Instrument

A pre-designed mixed type of questionnaire was used to collect the responses of the participants and documenting the results of culture and sensitivity test of the tissue samples.

3.10. Data collection tool: A predesigned mixed type of structured questionnaire followed during data collection.

3.11. Data processing and analysis

After collecting the data these were checked and rechecked for omission, inconsistencies and improbabilities. All questionnaires were checked immediately after completion by the researcher on site of data collection for missing fields. Obtained data were preserved in a secured place with strict confidentiality under direct responsibility of the thesis applicant.

Then checking of data was performed followed by editing, coding and entering into the computer. Data analysis was performed by statistical package for social science (SPSS), version-23. Appropriate statistical method was used after encoding data. Descriptive statistics was used to describe demographic data. Student's t-test was used to compare findings among male and female patients wherever appropriate. Result was presented with appropriate text, tables, and figures.

3.12. Ethical considerations

- This study was conducted after approval from Research cell and Ethical committee of Chattogram Veterinary and Animal Sciences University.
- Institutional clearance was obtained from Chattogram Diabetic General Hospital, Chattogram.
- Written informed consent was taken from all participants. The participants were not influenced or insisted to provide responses. Participants were briefed about purpose, procedure of the study in details implication of the study and detailed study related information was read out and explained in the local language from a printed hand out. All aspects including confidentiality and rights not to participate or withdrawal from the study

were specially communicated.

As per rule of Ethical Committee of CVASU-

1. Participation was voluntary.
2. Consent was obtained after a brief of the study in Bangla and technical terms were explained to all respondents wherever appropriate.
3. It was made clear to them that they are free to take part/ withdraw from the study at any stage.
4. All personal information will be kept confidential and will not be disclosed. Other responses will be used solely for the study purpose.
5. Interview was taken in a suitable time that was convenient to the respondents.
6. Refusal to take part or withdrawal from the study would not hamper in his/her treatment process.
7. The researcher did not intervene to establish any desired outcome.
8. The researcher informed the concerned authority when any problem or confusion arose.

3.13. Study procedure

A predesigned mixed type of structured questionnaire followed during data collection which contain personal information of the participants as well as information on grading of foot ulcer based on Wagner classification, size of ulcer, type and duration of diabetes mellitus, blood glucose level measurement, previous history of antibiotic use, risk factors of diabetes mellitus.

Data related to clinical findings such as neuropathy, vasculopathy, nephropathy, hypertension, and retinopathy were collected those were assessed based on their previous diagnosis. Written informed consent was taken before sample collection. Ulcer size was determined by multiplying the longest and widest diameters and expressed in centimeters squared. Ulcers were graded into following six categories according to the Wagner's Classification system (Turhan *et al.*,2013; Yahya *et al.*, 2016).

Figure 1.1: Different grades of diabetic foot ulcers: Grade-0, Grade-1, Grade-2, Grade-3, Grade-4, Grade-5

[Ref. Snaped taken by researcher herself in Chattogram diabetic general hospital]

Ulcer Grading:	**Description**
Grade 0	No ulcer but high risk foot
Grade 1	Superficial ulcer
Grade 2	Deep Ulcer, no bony involvement or abscess
Grade 3	Abscess with bony involvement
Grade 4	Localized gangrene e.g. toe, heel etc.
Grade 5	Extensive gangrene involving the whole foot

3.14. Bacterial Isolation

3.14.1. Sample collection procedure

Sterile cotton swab was used to collect sample from wound area. At first cleaned the surface of the surrounding ulcer using disinfectant. Then after rinsing the wound area

with normal saline sample was collected from the center of the diabetic wound. For each specimen two swabs were taken, one swab used for Gram staining and the other one inoculated on blood agar plates for isolating the microorganism Immediately the swab transferred into sterile tubes called Eppendorf tube having Stuart's transport medium. The tubes were trans-ported to the laboratory by immersion to maintain aseptic conditions. The samples were labeled in a proper way and immediately transported to the laboratory for investigation.

3.14.2. Microscopic study by staining method

Gram staining method was followed to study morphology and staining characters. Suspected colony from EMB agar was stained. The procedure is given below.

Sample from transport media was smeared on glass slide and fixed by heating

On smear crystal violate solution applied to stain for 2 minutes and then wash with running water

Few drops of Gram's iodine added act as a mordant and wait for 1 minute and then wash with running water again

Acetone alcohol was added for 3-4 seconds who act as a decolizer

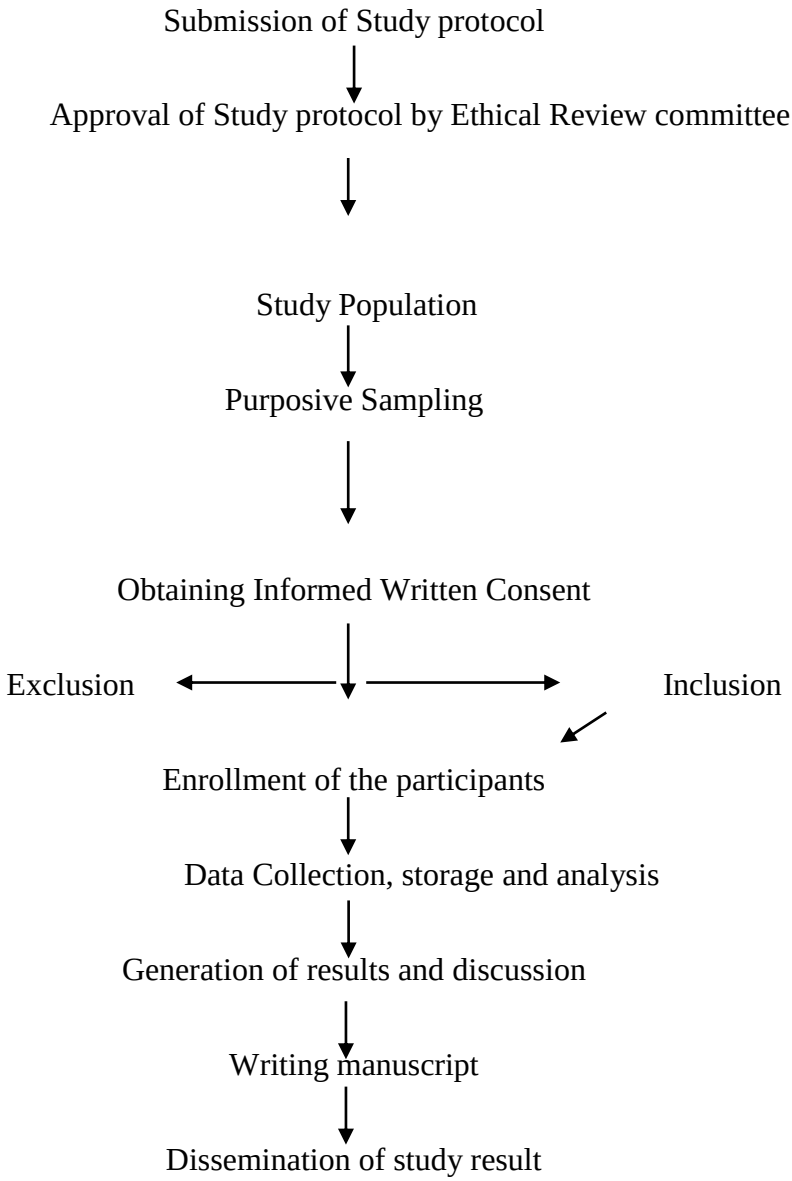
After washing with water, safranin was added as counter stain and allowed to stain for 1 minute

Then the slides were washed with water and dried in room temperature and then observed by microscope under 100X with emersion oil and characterization of bacteria recorded.

3.14.3. Bacteriological Investigation

The specimens were first inoculated onto blood agar and MacConkey agar media. The inoculated plates were incubated for 48 hours. The microorganisms were identified using standard biochemical procedure. The antimicrobial sensitivity of the organisms was performed by disc diffusion method according to the guidelines of the Clinical Laboratory Standards Institute (CLSI).

3.15. Study flow chart



3.16. Study Variables

3.16.1. General variables

1. Age
2. Sex
3. Occupation
4. Educational status
5. Socioeconomic status
6. Marital status
7. Height
8. Weight
9. Type of diabetes mellitus
10. Duration of Diabetes mellitus

3.16.2. Specific variables

1. Grading of ulcer
2. Size of the ulcer
3. Microorganism found in ulcer
4. Fasting Blood Sugar
5. Random Blood sugar
6. Gestational Diabetes Mellitus
7. Family history of Diabetes Mellitus
8. Body mass index
9. Lifestyle
10. Level of awareness
11. Diet
12. Hypertension
13. Cardiovascular disease

14. Cerebrovascular disease
15. Dyslipidemia
16. Retinopathy
17. Nephropathy
18. Neuropathy
19. Amputation
20. Vascular diseases

Chapter- 4: Results

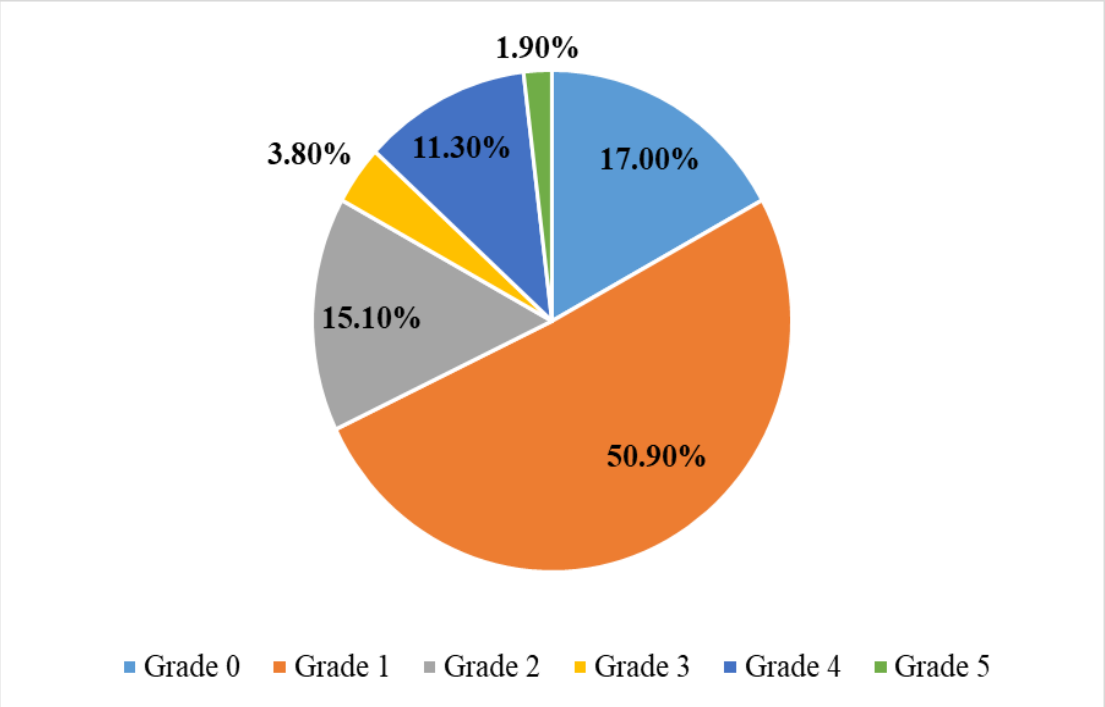


Figure 1.2: Distribution of grading of ulcers (n=106).

Figure 1.2 shows that out of 106 patients (100%), most of the patients (50.90%) were found grade 1 ulcer and lease of the patients (1.90%) were found grade 5 ulcer.

Table 1.1: Grading of ulcers in different age groups. (n=106)

		Grading of the Ulcer						Total
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
<35 Years	N	0	2	0	0	0	0	2
	%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	1.9%
35-39 Years	N	0	8	2	0	0	0	10
	%	0.0%	80.0%	20.0%	0.0%	0.0%	0.0%	9.4%
40-44 Years	N	0	2	2	0	2	0	6
	%	0.0%	33.3%	33.3%	0.0%	33.3%	0.0%	5.7%
45-49 Years	N	2	6	4	0	2	0	14
	%	14.3%	42.9%	28.6%	0.0%	14.3%	0.0%	13.2%
50-54 Years	N	2	10	0	0	2	2	16
	%	12.5%	62.5%	0.0%	0.0%	12.5%	12.5%	15.1%
55-59 Years	N	2	14	0	2	0	0	18
	%	11.1%	77.8%	0.0%	11.1%	0.0%	0.0%	17.0%
>59 Years	N	12	12	8	2	6	0	40
	%	30.0%	30.0%	20.0%	5.0%	15.0%	0.0%	37.7%
Total	N	18	54	16	4	12	2	106
	%	17.0%	50.9%	15.1%	3.8%	11.3%	1.9%	100.0%

Table 1.1 shows that out of 106 diabetic patients those were more than 59 years old had found foot ulcer (N= 40; 37.7%) more than others age group.

Table 1.2: Grading of ulcers in male and female participants. (n=106)

Sex		Grading of the Ulcer						Total
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
Male	N	8	34	8	0	8	2	60
	%	13.3%	56.7%	13.3%	0.0%	13.3%	3.3%	56.6%
Female	N	10	20	8	4	4	0	46
	%	21.7%	43.5%	17.4%	8.7%	8.7%	0.0%	43.4%
Total	N	18	54	16	4	12	2	106
	%	17.0%	50.9%	15.1%	3.8%	11.3%	1.9%	100.0%

Table 1.2 shows that male diabetic patients were having foot ulcer (N: 60; 56.6%) which is greater than female diabetic patients (N: 46, 43.4%).

Table 1.3: Grading of ulcers among patients involve with different occupations background with various socioeconomic status

Occupation & Socioeconomic status		Grading of the Ulcer					
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Unoccupied	N	2	10	4	2	6	0
	%	8.3%	41.7%	16.7%	8.3%	25.0%	0.0%
Sedentary work	N	4	28	6	2	6	2
	%	8.3%	58.3%	12.5%	4.2%	12.5%	4.2%
Laborious work	N	12	16	6	0	0	0
	%	35.3%	47.1%	17.6%	0.0%	0.0%	0.0%
Lower class	N	6	26	10	0	2	0
	%	13.6%	59.1%	22.7%	0.0%	4.5%	0.0%
Lower middle class	N	10	28	6	4	10	2
	%	16.7%	46.7%	10.0%	6.7%	16.7%	3.3%
Middle class	N	2	0	0	0	0	0
	%	100.0%	0.0%	0.0%	0.0%	0.0%	0.0%

Table 1.3 depicts that those diabetic patients involved with sedentary work developed foot ulcer more in comparison with those who involved with laborious work. This table shows that patients were found coming from lower middle class family (N: 60; 56.6%) more than other class.

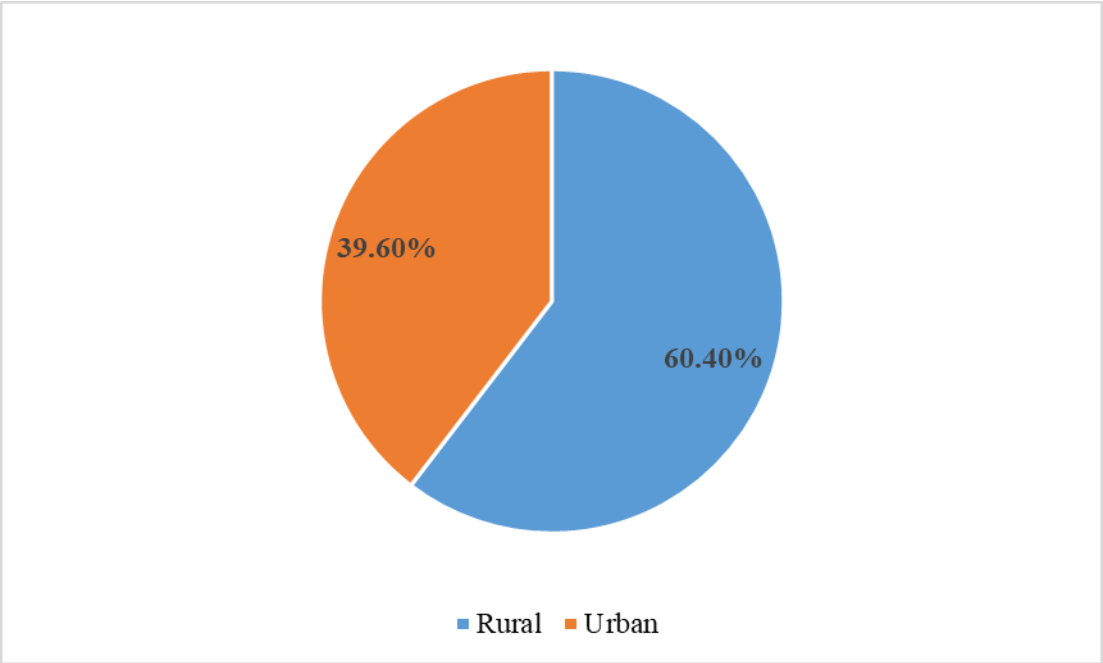


Figure 1.3: Habitants of the participants. (n = 106)

Figure 1.3 shows that patients were living in rural area (N: 64; 60.4%) more prone to develop diabetic foot ulcer than patients were living in urban area (N: 42; 39.6%).

Table 1.4: Relation between educational status with grading of ulcer. (n=106)

Educational Status		Grading of the Ulcer						Total
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
Uneducated	N	6	18	10	4	2	0	40
	%	15.0%	45.0%	25.0%	10.0%	5.0%	0.0%	37.7%
Up to Primary	N	8	10	0	0	0	0	18
	%	44.4%	55.6%	0.0%	0.0%	0.0%	0.0%	17.0%
Up to SSC	N	2	16	2	0	6	0	26
	%	7.7%	61.5%	7.7%	0.0%	23.1%	0.0%	24.5%
Up to HSC	N	2	6	0	0	0	2	10
	%	20.0%	60.0%	0.0%	0.0%	0.0%	20.0%	9.4%
Up to Graduation	N	0	2	4	0	2	0	8
	%	0.0%	25.0%	50.0%	0.0%	25.0%	0.0%	7.5%
Up to Post graduation	N	0	2	0	0	2	0	4
	%	0.0%	50.0%	0.0%	0.0%	50.0%	0.0%	3.8%
Total	N	18	54	16	4	12	2	106
	%	17.0%	50.9%	15.1%	3.8%	11.3%	1.9%	100.0%

Table 1.4 depicts that diabetic patients who were uneducated (N: 40; 37.7%) developed foot ulcer more than patients who were graduated (N:4; 3.8%). It reflects that educated patients aware well about diabetes and they take care of it but uneducated patients less aware about it.

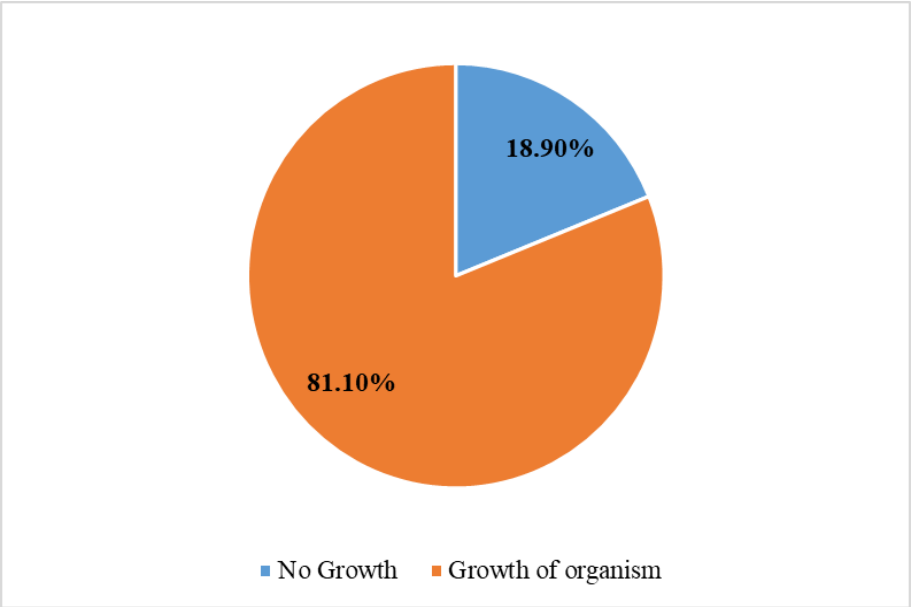


Figure 1.4: Growth of organisms in ulcers (n = 106)

Figure 1.4 shows that growth of organism was 81.10% isolated from diabetic foot ulcer on the contrary 18.90% was found no growth of organism.

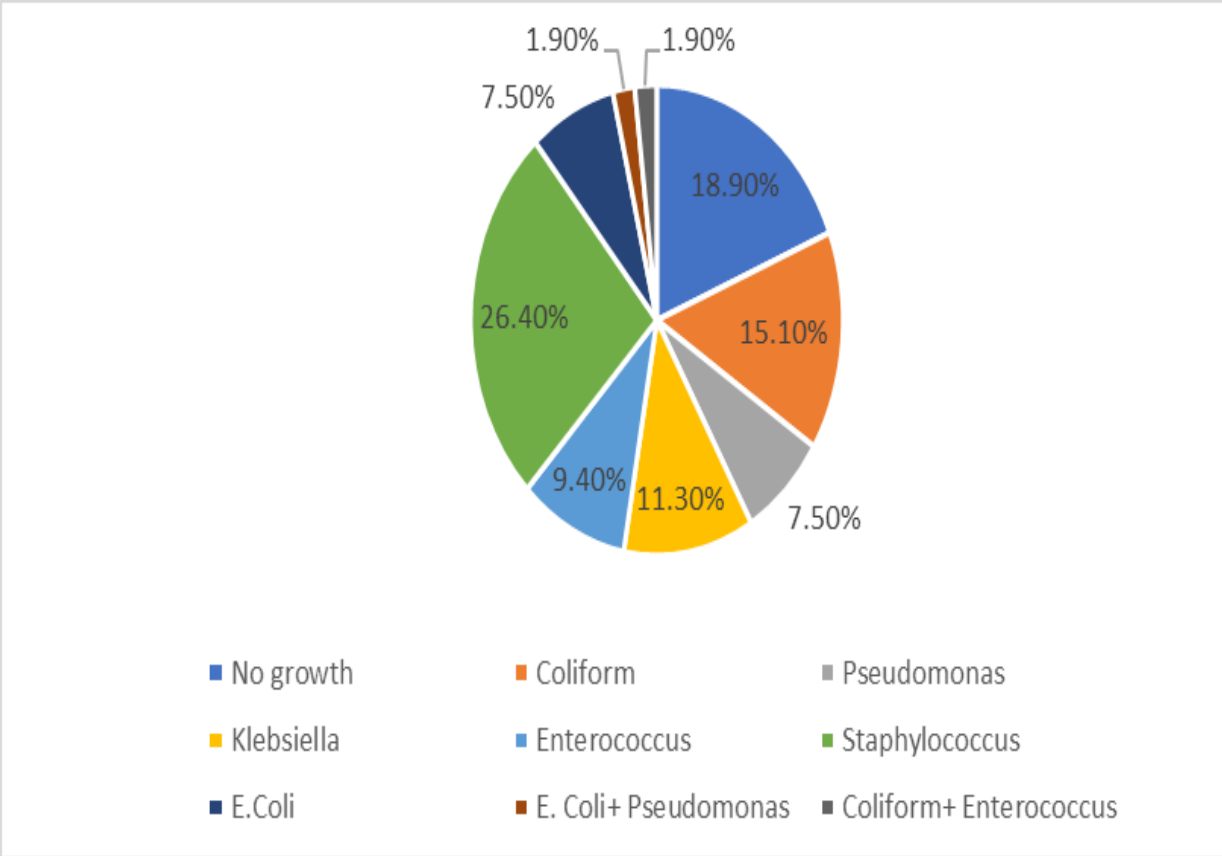


Figure 1.5: Growth of different organisms in ulcers (n = 106)

Figure 1.5 depicts that among 106 patients 86 patients (81.1%) with diabetic foot ulcer were found growth of organism whereas *Staphylococcus* found more (N: 28; 26.4%) than other organisms.

Table 1.5: Type of treatment was followed in diabetic patients

Type & Category of treatment		Grading of the Ulcer					
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5
Regular treatment	N	10	38	10	2	8	2
	%	14.3%	54.3%	14.3%	2.9%	11.4%	2.9%
Irregular treatment	N	6	14	2	2	4	0
	%	21.4%	50.0%	7.1%	7.1%	14.3%	0.0%
No treatment	N	2	2	4	0	0	0
	%	25.0%	25.0%	50.0%	0.0%	0.0%	0.0%
Oral hypoglycemic agent	N	2	10	4	0	0	2
	%	11.1%	55.6%	22.2%	0.0%	0.0%	11.1%
Insulin	N	2	12	0	0	2	0
	%	12.5%	75.0%	0.0%	0.0%	12.5%	0.0%
Both oral hypoglycemic & Insulin	N	2	10	4	0	0	2
	%	11.1%	55.6%	22.2%	0.0%	0.0%	11.1%

Table 1.5 shows that regular treatment was followed by most of the patients (N=70; 66.00%) after developing foot ulcer. It also describes the similar extend of use of oral hypoglycemic agents and combination of oral hypoglycemic agents with insulin (N=38; 35.80% in both conditions) by the subjects.

Table 1.6: Physical activity associated with grading of ulcer (n=106)

Physical Activity		Grading of the Ulcer						Total
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
Regular moderate physical activity	N	2	10	4	0	0	2	18
	%	11.1%	55.6%	22.2%	0.0%	0.0%	11.1%	17.0%
Irregular moderate physical activity	N	2	12	0	0	2	0	16
	%	12.5%	75.0%	0.0%	0.0%	12.5%	0.0%	15.1%
Mild physical activity	N	10	10	4	0	4	0	28
	%	35.7%	35.7%	14.3%	0.0%	14.3%	0.0%	26.4%
Sedentary (Neither exercise nor walk)	N	4	22	8	4	6	0	44
	%	9.1%	50.0%	18.2%	9.1%	13.6%	0.0%	41.5%
Total	N	18	54	16	4	12	2	106
	%	17.0%	50.9%	15.1%	3.8%	11.3%	1.9%	100.0%

Table 1.6 shows that diabetic patients who lead sedentary lifestyle (N: 44; 41.5%) were more prone to develop diabetic foot ulcer in comparison to patients who do regular physical activity (N: 18; 17.0%).

Table 1.7: Harmful personal habits in grading of ulcer (n=106)

Harmful factor		Grading of the Ulcer							Total
			Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
Smoking	H/O smoking	N	2	26	4	0	6	0	38
		%	5.3%	68.4%	10.5%	0.0%	15.8%	0.0%	35.8%
	No H/O smoking	N	16	28	12	4	6	2	68
		%	23.5%	41.2%	17.6%	5.9%	8.8%	2.9%	64.2%
Beetle Leaf	H/O beetle leaf chewing	N	10	34	10	2	2	0	58
		%	17.2%	58.6%	17.2%	3.4%	3.4%	0.0%	54.7%
	No H/O beetle leaf chewing	N	8	20	6	2	10	2	48
		%	16.7%	41.7%	12.5%	4.2%	20.8%	4.2%	45.3%
Total		N	18	54	16	4	12	2	106
		%	17.0%	50.9%	15.1%	3.8%	11.3%	1.9%	100.0%

Table 1.7 reflects that history of smoking in diabetic patients (N: 38; 35.8%) do not have any correlation to develop foot ulcer with those patients who do not smoke (N: 68; 64.2%).

Table 1.8: **Association between Knowledge and practice with Grading of ulcer (n=106)**

Knowledge & Practice		Grading of the Ulcer						Total
		Grade 0	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	
Appropriate knowledge & practice	N	0	4	0	0	0	0	4
	%	0.0%	100.0%	0.0%	0.0%	0.0%	0.0%	3.8%
Moderate knowledge & practice	N	4	8	4	0	0	2	18
	%	22.2%	44.4%	22.2%	0.0%	0.0%	11.1%	17.0%
Partial knowledge & irregular practice	N	6	18	0	2	10	0	36
	%	16.7%	50.0%	0.0%	5.6%	27.8%	0.0%	34.0%
Partial knowledge & occasional practice	N	6	22	6	2	2	0	38
	%	15.8%	57.9%	15.8%	5.3%	5.3%	0.0%	35.8%
No knowledge & no practice	N	2	2	6	0	0	0	10
	%	20.0%	20.0%	60.0%	0.0%	0.0%	0.0%	9.4%
Total	N	18	54	16	4	12	2	106
	%	17.0%	50.9%	15.1%	3.8%	11.3%	1.9%	100.0%

Table 1.8 shows that diabetic patients with appropriate knowledge and practice were found to have foot ulcer (N: 4; 3.8%) less than other patients with partial knowledge & irregular & occasional practice (N: 38; 35.8%).

Table 1.9: Frequency distribution of DSS score (n=106).

Risk Factors	Score	Frequency	Percentage (%)
Family History	1	34	32.08 %
	2	34	32.08%
	3	6	5.66%
	4	4	3.77%
	5	28	26.42%
BMI	1	10	9.43%
	2	54	50.94%
	3	38	35.85%
	4	4	3.77%
	5	0	0.00%
Diet	1	0	0.00%
	2	10	9.43%
	3	54	50.94%
	4	38	35.85%
	5	4	3.77%
Lifestyle	1	18	16.98%
	2	16	15.09%
	3	28	26.42%
	4	44	41.51%
	5	0	0.00%
Level of Awareness	1	4	3.77%
	2	18	16.98%
	3	36	33.96%
	4	38	35.85%
	5	10	9.43%
Associated Factors	1	0	0.00%
	2	26	24.53%
	3	30	28.30%
	4	50	47.17%
	5	0	0.00%

Table 1.9 shows that 26.42% participants had found with family history of DM among parents and at least one siblings. 41.51% participants had partial knowledge and occasional practice on DM.

Table 1.10: Descriptive values of risk factors among the respondents (n=106)

Risk factors	Minimum	Maximum	Mean	Std. Deviation
1. Family History	1	5	2.60	1.60
2. BMI	1	4	2.34	0.70
3. Diet	2	5	3.34	0.70
4. Lifestyle	1	4	2.92	1.11
5. Level of Awareness	1	5	3.30	0.98
6. Associated factors	2	4	3.23	0.82
DSS Score	**8**	**24**	**17.74**	**3.63**

Table depicts that among 106 respondents' diabetes sensitivity score based on risk factors was found between 8-24 and mean was 17.74.

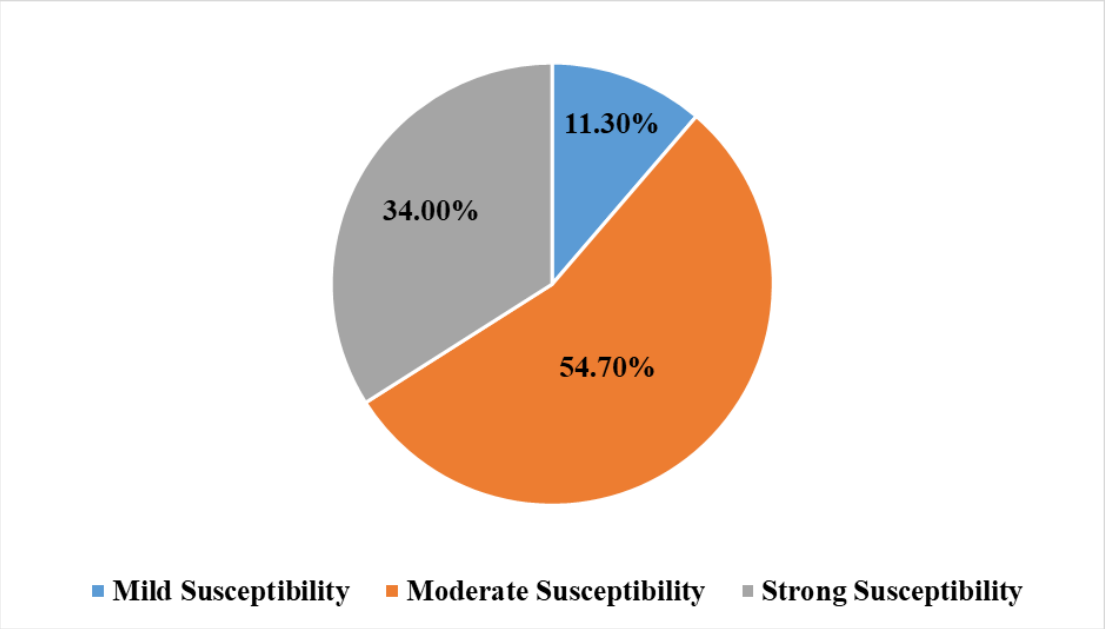


Figure 1.6: Level of diabetes sensitivity of the respondents (n=106)

According to Diabetes sensitivity score based on risk factors 11.30% respondents were found mild sensitivity, 34.00% were found moderate sensitivity and 54.70% were found strong sensitivity among 106 respondents.

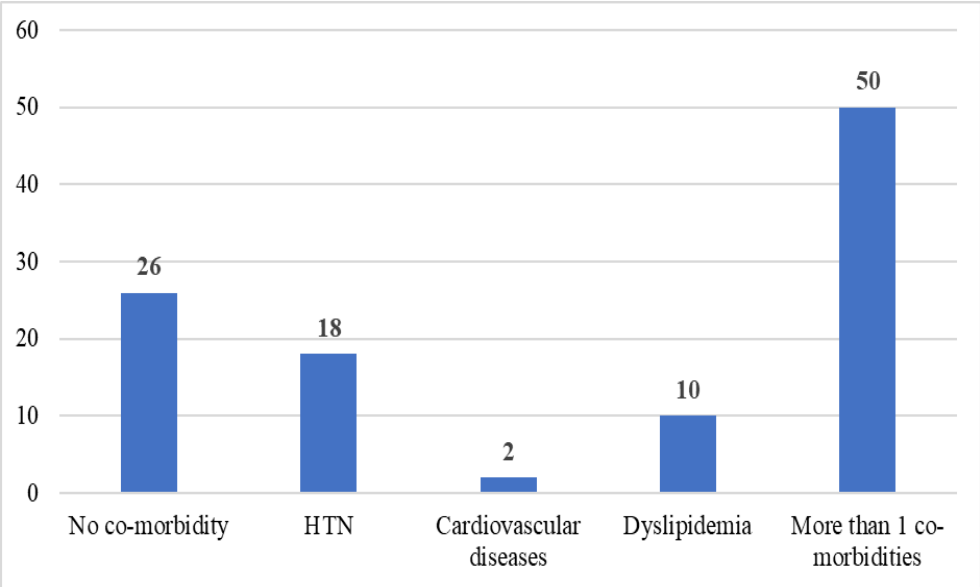


Figure 1.7 Co-morbidities among patients. (n = 106)

Figure 1.7 reflects that patients with more than one comorbidity were prone to develop foot ulcer (N: 50; 47.2%) in a greater number in comparison to patients have one comorbidity or no comorbidities.

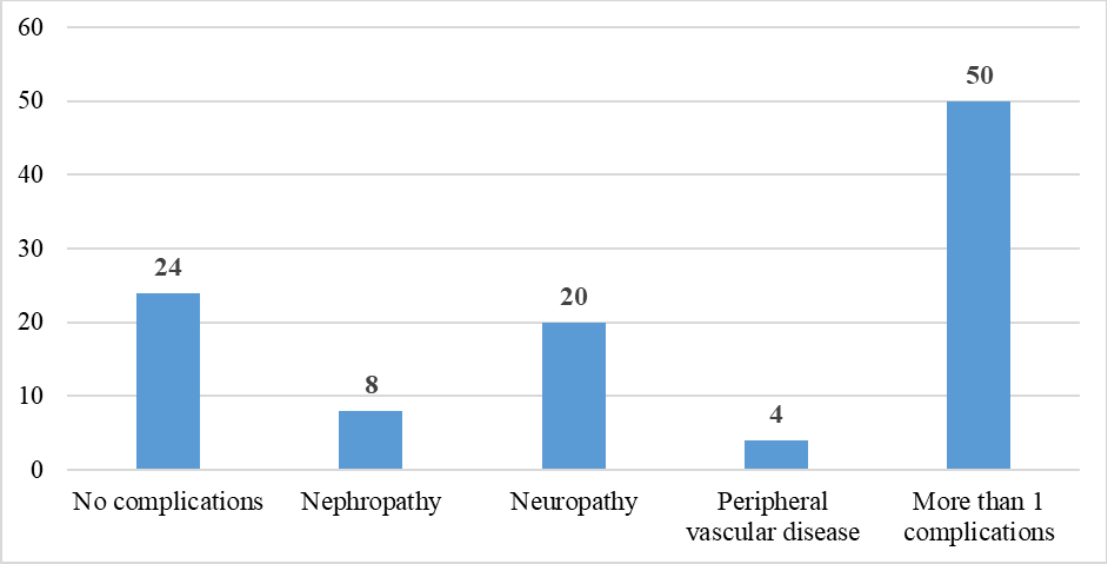


Figure 1.8: Complications found in patients. (n = 106)

Figure 1.8 shows that diabetic patients having foot ulcer were found with more than one complication (N: 50; 47.2%) in an immense number as opposed to patients have one or no complication.

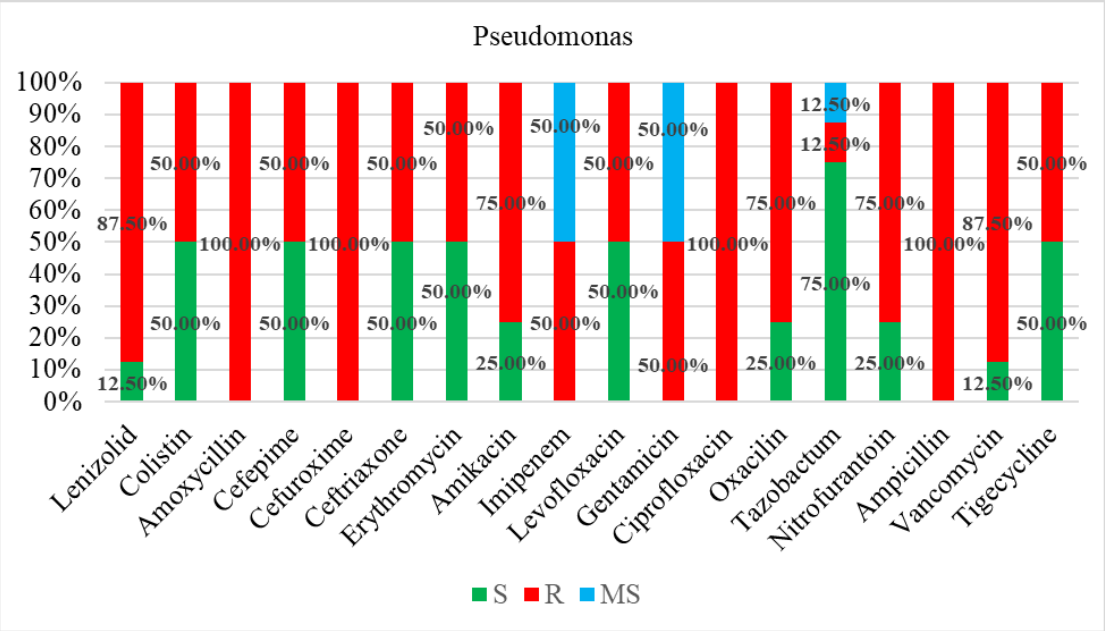


Figure 1.9: Antibiotic sensitivity of *Pseudomonas* observed in ulcer samples (n=106).

This figure depicts that *Pseudomonas* 100% resistant to Amoxycillin, Cefuroxime, Ciprofloxacin and Ampicillin. It shows 75% sensitive to Tazobactum.

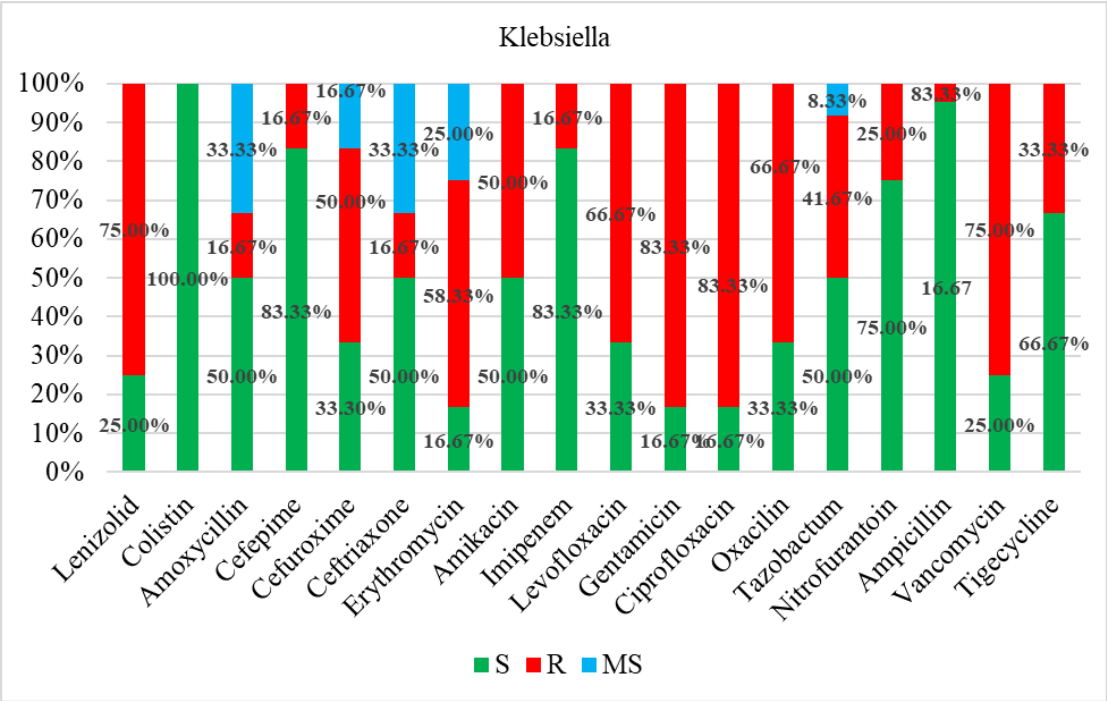


Figure 1.10: Antibiotic sensitivity of *Klebsiella* observed in ulcer samples (n=106).

Figure 1.10 shows that *Klebsiella* 100% sensitive to Colistin. To Ciprofloxacin and Gentamicin it shows 83.33% resistant.

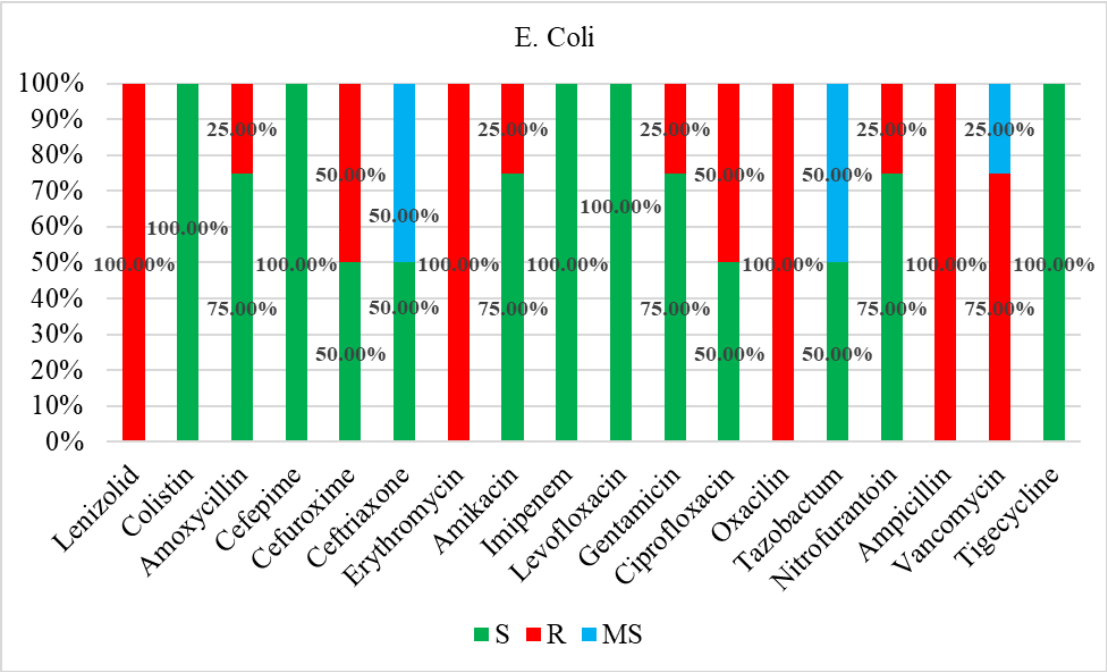


Figure 1.11: Antibiotic sensitivity of *E. Coli* observed in ulcer samples (n=106).

Figure 1.11 shows that *E. Coli* was found 100% resistant to Linezolid, Oxacillin, Ampicillin and 100% sensitive to colistin, Cefepime, Imipenem, levofloxacin and Tigecycline.

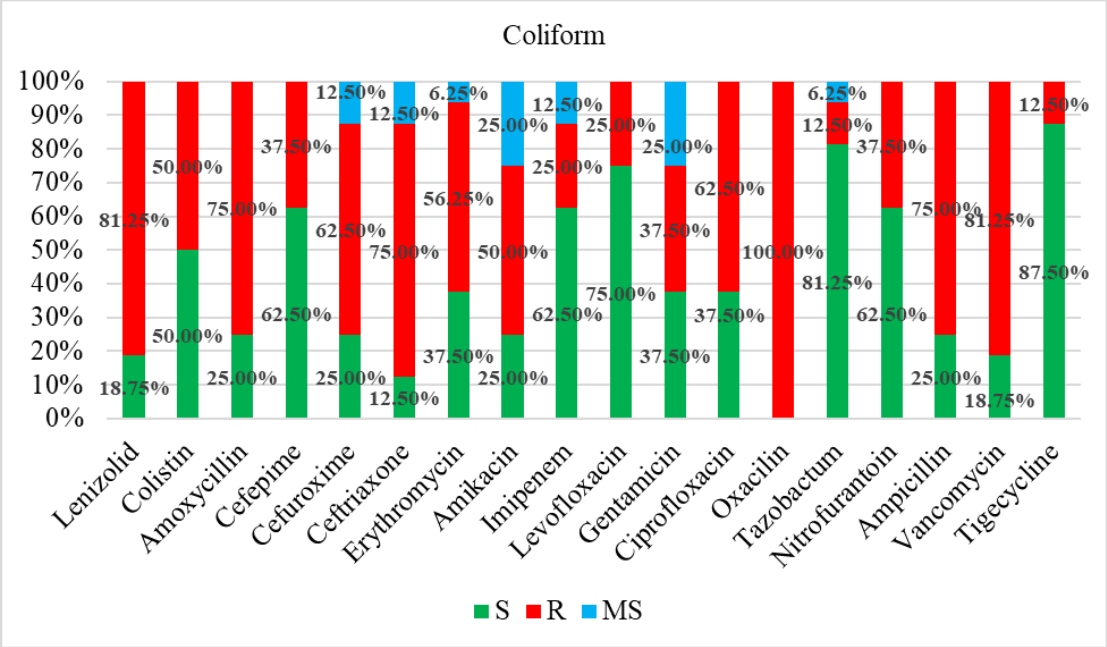


Figure 1.12: Antibiotic sensitivity of *Coliform* observed in ulcer samples (n=106).

Figure 1.12 reveals that *Coliform* was found 100% resistant to Oxacillin on the other hand 87.50% sensitive to Tigecycline.

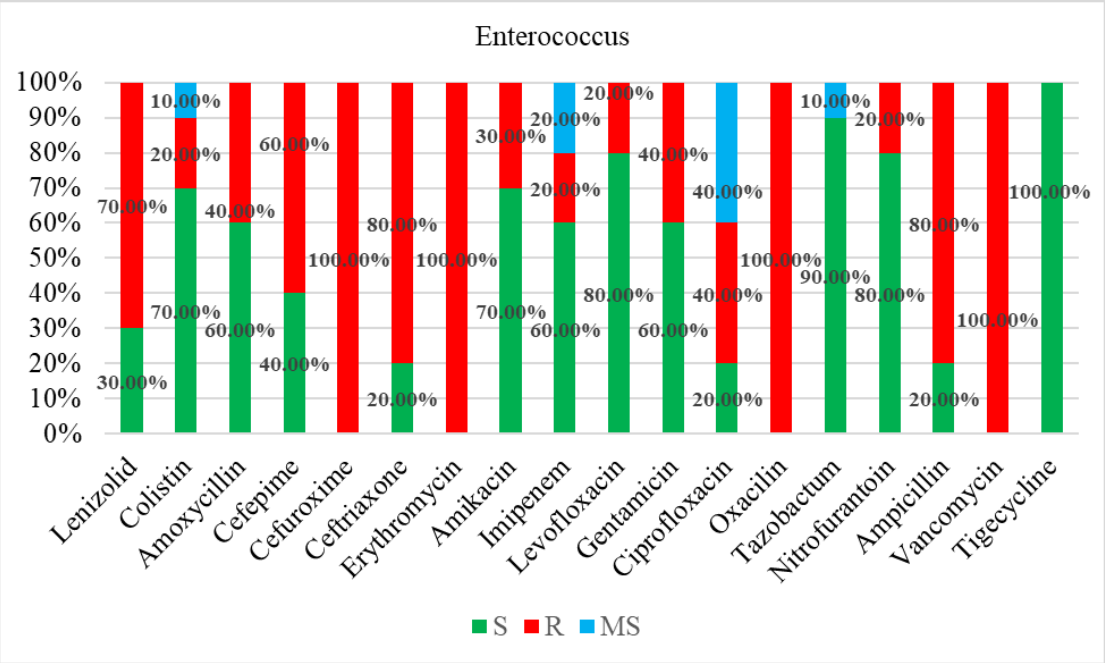


Figure 1.13: Antibiotic sensitivity of *Enterococcus* observed in ulcer samples (n=106).

Figure 1.13 reveals that *Enterococcus* was found 100% resistant to Cefuroxime, Erythromycin, Oxacillin and Vancomycin. On the other hand it was found 100% sensitive to Tigecycline.

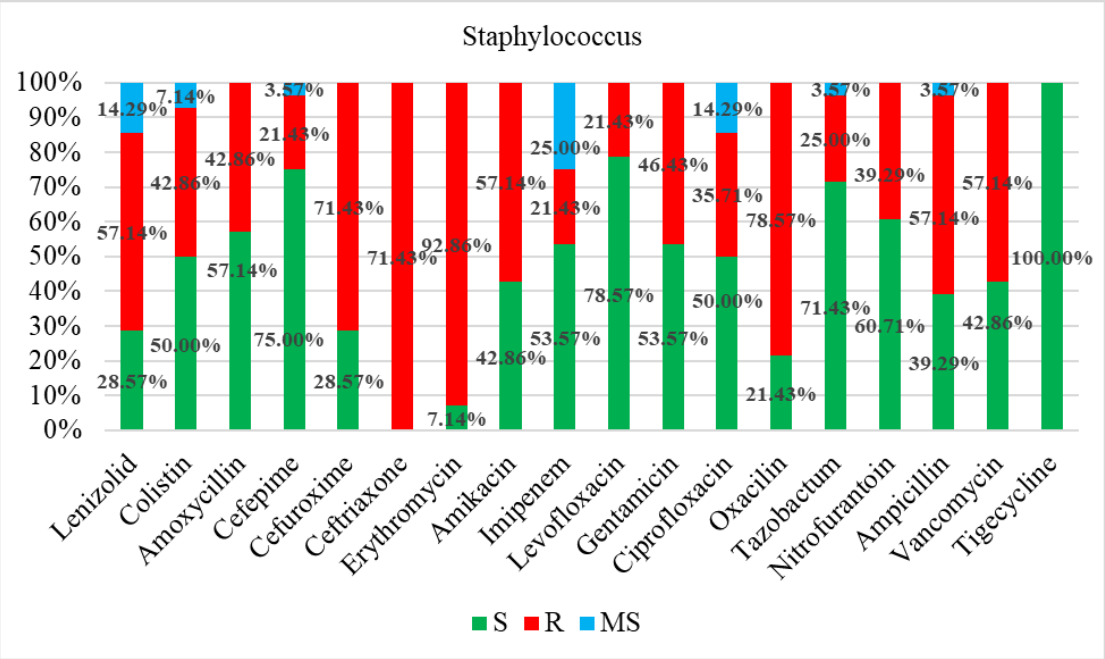


Figure 1.14: Antibiotic sensitivity of *Staphylococcus* observed in ulcer samples (n=106).

Figure 1.14 reveals that *Staphylococcus* showed 100% resistant to Ceftriaxone and 100% sensitive to Tigecycline.

Chapter-5: Discussion

The study was done with a total 106 diabetic patients with foot ulcer visiting diabetic hospital Chattogram.

The study found (Table-1.1) that the most of the respondents were having an age more than 59 years (40; 37.70%) followed by the age group having 55-59 years of age (18; 17.00%) while the lowest number of patients were from the relatively younger age having an age less than 35 years (2; 1.90%). Considering the highest number of participants based on grading of ulcer, the largest age groups were >59 years for Grade 0 (12), 55-59 years for Grade 1 (14), 50-54 years for Grade 2 (8), 50-54 years and 55-59 years for Grade 3 (2 in each group), >59 Years for Grade 4 (6) and 50-54 years for Grade 5 (2). Overall most of the patients were having an ulcer of Grade 1 (54; 50.90%) followed by Grade 0 (18; 17.00%) and Grade 2 (16; 15.10%) accordingly. Patients having ulcers with Grade 3 (4; 3.80%) or Grade 5 (2; 1.90%) were the least to be found.

Table 1.3 showed in this study, almost half (45.30%) of the subjects were found to do sedentary works who have ulcers more in comparison to subjects having exposure to laborious work (32.10%). Interestingly, the percentage of the unoccupied patients (22.60%) were close to the laborious group. It might be due to the frequent unemployment among the day laborers who had been usually work harder when having a job but remained unoccupied at the time of collecting data for this study. Similarly another study also found more patients with diabetes mellitus and foot ulcer living sedentary life style (Misra *et al.,* 2001). The larger numbers of patients with foot ulcers were hailing from rural areas (60.40%) might be due to the reason that people living in urban areas were more concerned about the early diagnosis of diabetes than the patients living in rural areas (Figure 1.3). On the contrary some studies shows that the prevalence of it highly seen in patient come from urban area than rural area (Mojtaba *et al.,* 2015; Mousumi *et al.,* 2016).

Level of education was found to play a pivotal role among the study participants in relation to the development of different grades of ulcers, which can be explained by the fact that educated patients were well aware of diabetes and took better care of diabetes. The increasing level of education beyond primary level (17.00%) seemed to

correspond inversely with the number of patients having ulcerative lesions. As per Table-5 there were gradually decreasing proportions of patients having education up to secondary level (24.50%) followed by higher secondary level (9.40%), graduation level (7.50%) and postgraduate level (3.80%). On the contrary, to expected findings, lower-middle-class people (60; 56.60%) were found to comprise the highest number of patients based on socio-economic status followed by lower class (44; 41.50%), whereas only 1.90% of the patients have belonged from middle socio-economic class (Table 1.4). It suggested the lack of access to health care facilities by lower socio-economic members of the society for diabetic care in their early stages.

We have seen in figure 1.4 and 1.5 there were six organisms found in culture done from the specimens collected from the patients' ulcer sites, namely *Coliform*, *Pseudomonas*, *Klebsiella*, *Enterococcus*, *Staphylococcus* and *E. Coli*. *Staphylococcus* was the most prevalent organism causing the ulcers in feet of the diabetic patients (28; 26.40%) followed by *Coliform* (16; 15.10%), *Klebsiella* (12; 11.30%), *Enterococcus* (10; 9.40%), *Pseudomonas* and *E. Coli* (8; 7.50%). Multiple organisms were found in four samples comprising *Coliform* and *Enterococcus*, and *Pseudomonas* and *E. Coli* (2; 1.90% in both groups). There was also a fair number of samples with no organisms growth (20; 18.90%). A number of studies have found that *Staphylococcus* is the main causative organism (Premanath *et al.*, 2019; Ramachandran *et al.*, 1986). We have also seen in our study that gram negative bacteria were the most frequently isolated organism. Similary Samir paul *et al.* (2009) found the gram negative bacteria is the most predominant organism (Rubinstein *et al.*, 1983). Alternatively few previous studies found that gram positive bacteria as the predominant organism in diabetic foot ulcer (Samir *et al.*, 2009; Sarita *et al.*, 2019; Sharma *et al.*, 2006). *Staphylococcus* aureus, *Escherichia coli, Staphylococcus, Proteus spp., Pseudomonas, Enterobacter spp., Morganella spp., Klebsiella spp., Citrobacter spp., Diphtheroid* were isolated from diabetic foot ulcer infection (Yahya *et al.*, 2016).
We have found that *Coliform, Klebsiella, Pseudomonas, E. Coli* were frequently isolated bacteria among gram negative bacteria. On the contrary, Gadepalli *et al.* found that Gram-negative bacteria (Proteus species, *E. Coli*, and *Pseudomonas* aeruginosa) were predominant strains (Turhan *et al.*, 2013). The regularity of treatment was involved moderately in influencing the ulcers' grading among the

patients as evident by an increased percentage of patients with Grade 2 ulcers (50.00%) in patients without treatment. According to Table 1.5 the treatment, whether regular or irregular, kept the grading of the ulcer lesions within Grade 0-1 (68.60% for Grade 0; 71.40% for Grade 1) in most cases. Type of hypoglycemic agents merely affected the outcome of the grading of ulcers among the study participants. There were equal numbers of patients found to use oral hypoglycemic agents alone and in combination with insulin. However, a slightly lower portion of the patients used insulin alone (22; 20.80%).

There was a noticeable effect of physical activity on the numbers of the subjects suffering from diabetic ulcers supported by patients' corresponding frequency with levels of physical activities. The correspondence of level of physical activity and steady increase in the number of patients was observed (Table 1.6) starting from the irregular moderate physical activity group (16; 15.10%), mild physical activity (28; 26.40%) and sedentary (Neither exercise nor walk) (44; 41.50%). Similarly Monica Matos *et al.* had done a systematic review which shows that exercise is a beneficial non-pharmacological treatment, delaying the usual course of diabetic peripheral neuropathy and delay skin damage and ulceration (Shiferaw *et al.,* 2016). Though exposure of the patients to smoking did not make a mentionable difference in their numbers to get foot ulcers the exposure to chewing of the beetle leaf (58; 54.70%) was associated with a higher number of patients with the lesion of interest of this study (Table 1.7). On the contrary Nan Xia *et al.* (2019) found smoking has association to develop diabetic foot ulcer and it’s progression.

As the previous understanding of the clinical outcome of the diabetic individuals based on the status of the knowledge on diabetes prevention or care and appropriate practice to control diabetes found to prevail in this study (Table 1.8). The study revealed that irregular and occasional practice to control diabetes led to a very close number of patients with diabetic ulcers (36; 34.00% & 38; 35.80%). Diabetic patients with appropriate knowledge and practice were found to have foot ulcer (N: 4; 3.8%). We have found that those patients have appropriate knowledge and practice about diabetes less prone to develop foot ulcer. Few studies also show that poor knowledge and practice about diabetic foot care more vulnerable to develop foot ulcer led to amputation also (Thaker *et al.,* 2013; Varma *et al.,* 1986).

Comorbidities more than one was responsible for approximately half of the ulcer cases in this study (50; 47.20%). Hypertension (18; 17.00%) was the most prevalent comorbidity besides the primary diabetic complication in the form of foot ulcer. A surprising number of patients reported no comorbidity when collecting data for this study might be a finding to explore by further research (Figure 1.6). Herbert F Jelinek et. al. showed that hypertension was the most common comorbidities found in diabetic patients and also found other comorbidities like dyslipidemia, obesity (Waqas *et al.,* 2016)

Since diabetic ulcer was already a complication present in all the study subjects, there was also at least one other complication (i.e. nephropathy, neuropathy, peripheral vascular disease) present in most of the patients (82; 77.40%) (Figure 1.7). Unfortunately, many patients were further burdened with more than one complication (50; 47.20%). Diabetes mellitus and it's chronic complications have become gradually common. Few studies found that complications like retinopathy, nephropathy, neuropathy etc. developed chronically with the increasing duration of diabetes mellitus (Waqas *et al.,* 2016).

In this study 18 antibiotics were used. Some of the standard antibiotics are: Amikacin, Ampicillin, Amoxycillin, Colistin, cefepime, cefuroxime, Ceftriaxone, Ciprofloxacin, Levofloxacin, Erythromycin, Gentamycin, Imipenem, Linezolid, Oxacillin, Vancomycin, Tazobactum, Nitrofurantoin.

From Figure 1.9 to Figure 1.14 we have found that *Coliform*+ *Enterococcus* (N: 2; 100%); *Staphylococcus* (N:8;28.6%) and *Coliform* (N: 2; 12.5%) were sensitive to linezolid but 100% *E. Coli* (N: 8; 100.0%); largest number of *Coliform* (N: 13; 81.3%) & *Pseudomonas* (N: 7; 87.5%); *Klebsiella* (N: 9; 75.0%); *Enterococcus* (N: 7; 70.0%) and *Staphylococcus* (N: 16; 57.1%) were resistant to it. Small number of *Staphylococcus* (N; 4; 14.3%) were medium sensitive to Linezolid.

Largest number of Bacteria from both gram positive and gram-negative bacteria were sensitive to Colistin and a smaller number of bacteria were medium sensitive & resistant to Colistin. Among gram positive bacteria *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N: 12; 42.9%) and among gram negative bacteria *Coliform* (N: 8; 50.0%); *Pseudomonas* (N: 4; 50.0%) were sensitive to Colistin. Among gram positive bacteria *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N: 14; 50.0%) and among gram negative bacteria *E. Coli* (N: 8; 100.0%); *Klebsiella* (N: 12; 100.0%) *Coliform*

(N: 8; 50.0%); *Pseudomonas* (N: 4; 50.0%) were resistant to Colistin. It shows that gram positive bacteria *Enterococcus* (N: 6; 60.0%); *Staphylococcus* (N: 16; 57.1%) and gram-negative bacteria like *Coliform* (N: 4; 25.0%); *Klebsiella* (N; 6; 50.0%); *E. Coli* (N: 6; 75.0%) were sensitive to amoxycillin. On the other hand, *Enterococcus* (N: 4; 40.0%); *Staphylococcus* (N: 12; 42.9%) and gram-negative bacteria *Coliform* (N: 12; 75.0%); *Pseudomonas* (N: 8; 100.0%); *Klebsiella* (N; 2; 16.7%); *E. Coli* (N: 2; 25.0%) were resistant to amoxycillin. Only *Klebsiella* (N: 4; 33.3%) was medium sensitive to Amoxycillin. Largest number of both gram-positive bacteria *Enterococcus* (N: 4; 40.0%); *Staphylococcus* (N: 21; 75.0%) and gram-negative bacteria like *Coliform* (N: 10; 62.5%); *Klebsiella* (N; 10; 83.3%); *E. Coli* (N: 8; 100.0%) were sensitive to amoxycillin. On the other hand, gram-positive bacteria *Enterococcus* (N: 6; 60.0%); *Staphylococcus* (N: 6; 21.4%) and gram-negative bacteria *Coliform* (N: 6; 37.5%); *Pseudomonas* (N: 4; 50.0%); *Klebsiella* (N; 2; 16.7%) were resistant to amoxycillin.

Largest number of both gram-positive bacteria *Enterococcus* (N: 10; 100.0%); *Staphylococcus* (N: 20; 71.4%) and gram-negative bacteria like *Pseudomonas* (N: 8; 100.0%); *Coliform* (N: 10; 62.5%); *Klebsiella* (N; 6; 50.0%); *E. Coli* (N: 4; 50.0%) were resistant to cefuroxime in comparison to only *Staphylococcus* (N: 8; 28.6%) and gram-negative bacteria *Coliform* (N: 4; 25.0%); *Klebsiella* (N; 4; 33.3%) and *E. Coli* (N: 4; 50.0%) were sensitive to cefuroxime. Besides only *Coliform* (N: 2; 12.5%); *Klebsiella* (N; 2; 16.7%) were found medium sensitive to cefuroxime.

A large number of both gram-positive bacteria *Enterococcus* (N: 8; 80.0%); *Staphylococcus* (N: 20; 71.4%) and gram-negative bacteria like *Pseudomonas* (N: 4; 50.0%); *Coliform* (N: 12; 75.0%); *Klebsiella* (N; 2; 16.7%) were resistant to Ceftriaxone whereas only *Staphylococcus* (N: 8; 28.6%); *Enterococcus* (N; 2; 20.0%) and gram-negative bacteria *Coliform* (N: 2; 12.5%); *Klebsiella* (N; 6; 50.0%) and *E. Coli* (N: 4; 50.0%) were sensitive to Ceftriaxone. Alongside *Coliform* (N: 2; 12.5%); *Klebsiella* (N; 4; 33.3%) and *E. Coli* (N; 4; 50.0%) were found medium sensitive to Ceftriaxone.

It depicts that a small number of both gram-positive bacteria *Staphylococcus* (N: 2; 7.1%) and gram-negative bacteria *Coliform* (N: 6; 37.5%); *Pseudomonas* (N: 4; 50.0%) and *Klebsiella* (N; 2; 16.7%) were sensitive to *Erythromycin* on the contrary a large number of both gram-positive bacteria *Enterococcus* (N: 10; 100.0%);

Staphylococcus (N: 26; 92.9%) and gram-negative bacteria like *E. Coli* (N: 8; 100.0%); *Pseudomonas* (N: 4; 50.0%); *Coliform* (N: 9; 56.3%) and *Klebsiella* (N; 7; 58.3%) were resistant to Erythromycin.

Alongside *Coliform* (N: 1; 6.3%) and *Klebsiella* (N; 3; 25.0%) were found medium sensitive to Erythromycin. Gram positive bacteria *Enterococcus* (N: 7; 70.0%); *Staphylococcus* (N: 12; 42.9%) and gram-negative bacteria like *Coliform* (N: 4; 25.0%); *Klebsiella* (N; 6; 50.0%); *E. Coli* (N: 6; 75.0%) and *Pseudomonas* (N: 2; 25.0%) were sensitive to Amikacin. On the other hand, gram positive bacteria *Enterococcus* (N: 3; 30.0%); *Staphylococcus* (N: 16; 57.1%) and gram-negative bacteria *Coliform* (N: 8; 50.0%); *Pseudomonas* (N: 6; 75.0%); *Klebsiella* (N; 6; 50.0%) and *E. Coli* (N: 2; 25.0%) were resistant to Amikacin.

Largest number of both gram-positive bacteria *Enterococcus* (N: 6; 60.0%); *Staphylococcus* (N: 15; 53.6%) and gram-negative bacteria like *Coliform* (N: 10; 62.5%); *Klebsiella* (N; 10; 83.3%); *E. Coli* (N: 8; 100.0%) were sensitive to Imipenem. On the other hand, a small number of both gram-positive bacteria *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N: 6; 21.4%) and gram-negative bacteria *Coliform* (N: 4; 25.0%); *Pseudomonas* (N: 4; 50.0%); *Klebsiella* (N; 2; 16.7%) were resistant to Imipenem. Moreover, *Coliform* (N:2;12.5%); *Pseudomonas* (N: 4;5 0.0%); *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N:7; 25.0%) were found medium sensitive to Imipenem.

Largest number of both gram-positive bacteria *Enterococcus* (N: 8; 80.0%); *Staphylococcus* (N: 22; 78.6%) and gram-negative bacteria like *Coliform* (N: 12; 75.0%); *Klebsiella* (N; 4; 33.3%); *E. Coli* (N: 8; 100.0%) were sensitive to Levofloxacin. On the other hand, a small number of both gram-positive bacteria *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N: 6; 21.4%) and gram-negative bacteria *Coliform* (N: 4; 25.0%); *Pseudomonas* (N: 4; 50.0%); *Klebsiella* (N; 8; 66.7%) were resistant to Levofloxacin. We found that gram positive bacteria *Enterococcus* (N: 6; 60.0%); *Staphylococcus* (N: 15; 53.6%) and gram-negative bacteria like *Coliform* (N: 6; 37.5%); *Klebsiella* (N; 2; 16.7%) and *E. Coli* (N: 6; 75.0%) were sensitive to Gentamicin. On the other hand, gram positive bacteria *Enterococcus* (N: 4; 40.0%); *Staphylococcus* (N: 13; 46.4%) and gram-negative bacteria *Coliform* (N: 6; 37.5%); *Pseudomonas* (N: 4; 50.0%); *Klebsiella* (N; 10; 83.3%) and *E. Coli* (N: 2; 25.0%) were resistant to Gentamicin.

A small number of bacteria *Coliform* (N: 4; 25.0%) and *Pseudomonas* (N: 4; 50.0%) were found medium sensitive to Gentamicin. Many gram-negative bacteria like *Coliform* (N: 10; 62.5%); *Klebsiella* (N; 10; 83.3%); *E. Coli* (N: 4; 100.0%); *Pseudomonas* (N: 8; 100.0%) were resistant to Ciprofloxacin as well as gram-positive bacteria *Enterococcus* (N: 4; 40.0%); *Staphylococcus* (N: 10; 35.7%) had found resistant to Ciprofloxacin. On the other hand, gram-negative bacteria like *Coliform* (N: 6; 37.5%); *Klebsiella* (N; 2; 16.7%); *E. Coli* (N: 4; 50.0%) and gram-positive bacteria *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N: 14; 50.0%) were found sensitive to Ciprofloxacin. *Enterococcus* (N: 4; 40.0%); *Staphylococcus* (N: 4; 14.3%) had found medium sensitive to Ciprofloxacin. Considering 2 groups of quinolones, a large number of bacteria were found sensitive to levofloxacin in compare to Ciprofloxacin.

Greatest number of both gram-positive bacteria *Enterococcus* (N:10; 100.0%); *Staphylococcus* (N:22; 78.6%) and gram-negative bacteria like *Coliform* (N: 16; 100.0%); *Klebsiella* (N; 8; 66.7%); *E. Coli* (N: 8; 100.0%) were found resistant to Oxacilin. On contrary a small number of both gram-positive bacteria *Staphylococcus* (N: 6; 21.4%) and gram-negative bacteria *Pseudomonas* (N: 2; 25.0%); *Klebsiella* (N:4; 33.3%) were resistant to Oxacilin. Most of the organism found in diabetic foot ulcer resistant to Oxacillin.

A large number of both gram-positive bacteria *Enterococcus* (N: 9; 90.0%); *Staphylococcus* (N: 20; 71.4%) and gram-negative bacteria like *Pseudomonas* (N: 6; 75.0%); *Coliform* (N: 13; 81.3%); *Klebsiella* (N; 6; 50.0%) and *E. Coli* (N: 4; 50.0%) were found in diabetic foot ulcer sensitive to Tazobactum whereas gram positive bacteria *Staphylococcus* (N: 7; 25.0%) and gram-negative bacteria *Coliform* (N: 2; 12.5%); *Klebsiella* (N; 5; 41.7%) and *Pseudomonas* (N: 1; 12.5%) were resistant to Tazobactum. Alongside *Coliform* (N: 1; 6.3%); *Klebsiella* (N; 1; 8.3%); *Pseudomonas* (N:1; 12.5%); *Staphylococcus* (N: 1; 3.6%) and *E. Coli* (N; 4; 50.0%) were found medium sensitive to Tazobactum.

A large number of both gram-positive bacteria *Enterococcus* (N: 8; 80.0%); *Staphylococcus* (N: 17; 60.7%) and gram-negative bacteria like *Pseudomonas* (N: 2; 25.0%); *Coliform* (N: 10; 62.5%); *Klebsiella* (N; 9; 75.0%) and *E. Coli* (N: 6; 75.0%) were found in diabetic foot ulcer sensitive to Nitrofurantoin. On the contrary a small number of gram positive bacteria *Staphylococcus* (N: 11; 39.3%) and gram-negative

bacteria *Coliform* (N: 6; 37.5%); *Klebsiella* (N; 3; 25.0%); *Pseudomonas* (N: 6; 75.0%) and *E. Coli* (N: 2; 25.0%) were resistant to Nitrofurantoin. Most of the organism were found in Diabetic foot ulcer sensitive to Nitrofurantoin except *Pseudomonas* were resistant. Gram positive bacteria *Enterococcus* (N: 2; 20.0%); *Staphylococcus* (N: 11; 39.3%) and gram-negative bacteria like *Coliform* (N: 4; 25.0%); *Klebsiella* (N; 2; 16.7%) were sensitive to Ampicillin. On the other hand, *Enterococcus* (N: 8; 80.0%); *Staphylococcus* (N: 16; 57.1%) and gram-negative bacteria *Coliform* (N: 12; 75.0%); *Pseudomonas* (N: 8; 100.0%); *Klebsiella* (N; 10; 83.3%); *E. Coli* (N: 8; 100.0%) were resistant to amoxycillin. Most of the organism were found in diabetic foot ulcer resistant to different types of penicillin like Ampicillin, Amoxycillin and Oxacillin.

A large number of both gram-positive bacteria *Enterococcus* (N:10; 100.0%); *Staphylococcus* (N:16; 57.1%) and gram-negative bacteria like *Coliform* (N: 13; 81.3%); *Klebsiella* (N; 9; 75.0%); *E. Coli* (N: 6; 75.0%) and *Pseudomonas* (N: 7; 87.5%) were found resistant to Vancomycin. On the contrary a small number of both gram-positive bacteria *Staphylococcus* (N: 12; 42.9%) and gram-negative bacteria *Pseudomonas* (N: 1; 12.5%); *Klebsiella* (N:3; 25.0%) and *Coliform* (N:3; 18.8%) were sensitive to Vancomycin. *E. Coli* (N:2; 25.0%) was found medium sensitive to Vancomycin.

A largest number of both gram-positive bacteria *Enterococcus* (N:10; 100.0%); *Staphylococcus* (N:28; 100.0%) and gram-negative bacteria like *Coliform* (N: 14; 87.5%); *Klebsiella* (N; 8; 66.7%); *E. Coli* (N: 8; 100.0%) and *Pseudomonas* (N:4; 50.0%) were found in diabetic foot ulcer sensitive to Tigecycline. On the other hand, no organism found in diabetic foot ulcer were resistant to Tigecycline. Besides this, *Coliform* (N:2;12.5%), *Pseudomonas* (N:4; 50.0%) and *Klebsiella* (N: 4; 33.3%) were found medium sensitive to Tigecycline.

Overall, we have found that gram positive bacteria both *Staphylococcus* and *Enterococcus* 100% sensitive to only Tigecycline among 18 antibiotics alternatively *Enterococcus* showed 100 % resistant to Cefuroxime, Erythromycin and Oxacillin. Among gram negative bacteria, *E. Coli* showed 100% sensitive to Colistin, Cefepime, Imipenem, Levofloxacin, Tigecycline in addition *Klebsiella* showed 100% sensitive to Colistin only. On the other hand, *Pseudomonas* and *Coliform* showed no 100% sensitivity to any antibiotic. Unfortunately, *E. Coli* showed 100% resistant to

Linezolid, Erythromycin, Oxacillin and Ampicillin similarly *Pseudomonas* showed 100% resistant to Amoxycillin, Cefuroxime, Ciprofloxacin, Ampicillin and *Coliform* 100% resistant to Oxacillin. Finally we found bacteria both gram positive and gram negative isolated from foot ulcer infection didn't show any resistance to one antibiotic Tigecycline. High levels of resistance to ciprofloxacin, cotrimoxazole, amikacin, gentamicin and cephalosporins were found in all isolated organisms. Only Imipenem was the most effective agent against all Gram-negative organisms (Varma *et al.*, 1986).

All the Gram-positive bacteria showed good sensitivity to most of the antibiotics. *Enterococcus* faecalis showed lesser sensitivity for the antibiotics. The Gram-negative bacteria showed good activity against amikacin, cephalexin, amoxicillin, gentamycin, ofloxacin, piperacillin-tazobactum, ticarcillin-clavulanic acid combinations (Waqas *et al.,* 2016). Many of the organisms showing resistance to Penicillin had been reported (Turhan *et al.*, 2013). As for *Staphylococcus* spp., linezolid and vancomycin were the most effective antibiotics (Yahya *et al.,* 2016) but we have found that *Staphylococcus* was resistant to Linezolid and Vancomycin in percentage of 57.1 and 57.1, respectively. In another study isolated *Staphylococcus* sp. were resistant to Vancomycin . Isolated *Pseudomonas* and *E. Coli* also showed resistance to Vancomycin (Varma *et al.*, 1986). Similarly we also found that *Pseudomonas* 87.5% and *E. Coli* 75.0% were resistant to vancomycin.

The high rates of antibiotic resistance had been found in our study may be due to such factors including hospitalization, frequently use of broad-spectrum antibiotics, irrational use of antibiotics, and also may be due to presence of resistant gene.

Chapter-6: Conclusions

6.1. Conclusion

- High rates of antibiotic resistance to most of the organisms isolated from diabetic foot ulcer have been seen in this study. Most of the organisms were found resistant to penicillin group of drugs like Ampicillin, Amoxycillin, Oxacillin. The increasing rate of antibiotic resistance may lead to cause severe complication like amputation from a small size foot ulcer.
- *Staphylococcus* is the most predominant organism isolated from diabetic foot ulcer. Isolated *Staphylococcus* and *Enterococcus* were found sensitive to Tigecycline.
- A largest number of participants were hailing from rural area might be due to the reason that people living in urban areas were more concerned about the early diagnosis of diabetes than the patients living in rural areas.
- Diabetic patients should be educated about foot care and aware them about the severe complications of foot ulcer. We should also keep in mind that appropriate antibiotic choice is an important issue to reduce the complication of diabetic foot ulcer.

6.2. Limitations of the study

Although optimum care had been tried by the researcher in every steps of this study, still there were some limitations. The results were be interpreted in the light of the following limitations.

- Due to this sudden pandemic COVID-19 situation sample collection was limited.
- Since the sampling was done purposively there could be some selection bias.
- The study place was in Chattogram Diabetic General Hospital for which the sample may not be representative of the problems in whole Bangladesh.
- Due to time constrain and COVID-19 situation PCR was not done and whether any antibiotic resistant gene present or not it could not be found out.
- All type of Antibiotics available in local market was not seen sensitivity due to budget constrain.

Chapter-7: Recommendations and Future perspectives

Despite several limitations, this study provided data on complications and comorbidities of diabetes mellitus as well as antibiotic sensitivity pattern. This was the first study to explore the patterns in Chattogram, Bangladesh which will help in the future to treat the patients with diabetic foot ulcer.

Although this study has used pre-tested and predesigned data collection instrument and detailed measures to analyze the obtained data, the findings can be thereby used in future studies yet the researchers should view the findings as provisional and approximate.

In the light of this research work, the researcher recommended the followings:

- Physician should do culture and sensitivity routinely before prescribing any antibiotic to diabetic patients with foot ulcer to prevent further severe complications like foot amputation.
- To alleviate this situation and reduce the rate of amputation, clinicians should prescribe antibiotics rationally, timely, and sufficiently in a proper way.
- As the cost of culture and sensitivity investigation is high every patient cannot afford this. If the test cost is reduced maximum patients can perform the test.
- Further exploratory studies are required to evaluate the presence of any antibiotic resistant gene.
- A nationwide randomized study should be done to explore the scenario in Bangladesh.

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Chapter – 8: Appendices

Appendix A: Schedule of works

Activities	1st month	2nd month	3rd month	4th month	5th month	6th month
Ethical Approval	■					
Designing the study	■					
Sample and Data collection, Microbiological study		■	■			
Data analysis and result generation				■		
Writing the manuscript					■	■
Submission and Presentation of thesis						■

Appendix B: Informed Consent form (English Version)

Title of the Study: "**Quantification & scaling of risk factors of diabetes mellitus and isolation of microorganism from diabetic foot ulcer: a cross sectional study.**"

Date and Time of Interview-

Name-

Address-

I know all the steps involved in this research. I am well explained about the purpose, procedure (aseptic condition will be maintained during sample collection and no other invasive procedure will be done) and fate of the research data and also informed about how much time it will need to respond. I have understood the matter very well and I am also satisfied about the way of explanation. I have provided with a written information sheet with details of the study.

I have clearly understood that by participating in this research, not only myself, other patients also will be benefited. During any stage of the research I can withdraw my consent and this decision will not hamper on my treatment procedure.

I have also clearly understood that during research activity if I have any query or problem the researcher will be there to resolve the issue. I also know that my information will be kept with strict confidentiality and anonymity. I am aware that only the results of the study, not the personal information will be published.

I have read the paper explaining the research thoroughly and agreed to participate in the study as respondent with profound understanding.

_____ _____

Signature of the participant with date Signature of the researcher with date

Appendix B: Informed Consent form (Bengali version)

সম্মতিপত্র

Title: "Quantification & scaling of risk factors of diabetes mellitus and isolation of microorganism from diabetic foot ulcer: a cross sectional study".

তারিখঃ

নামঃ বয়সঃ

ঠিকানাঃ

এই গবেষণায় যে কার্যক্রমগুলো করা হবে আমি সে সম্পর্কে অবগত আছি। এ পদ্ধতিগুলো সম্পর্কে আমাকে ব্যাখ্যা করা হয়েছে যেমন,

১) রোগের ইতিহাস জানা এবং শারীরিক পরীক্ষা করা।

২) পায়ের ক্ষত থেকে পুঁজ পরীক্ষা করা।

৩) রক্তের গ্লুকোজ পরিমাপ করা।

এবং কোন ধরণের অসুবিধা হতে পারে তাও বলা হয়েছে। আমি এই বিষয়ে সন্তুষ্ট।

আমি বুঝেছি যে এই গবেষণায় অংশগ্রহণের মাধ্যমে আমি সরাসরি উপকৃত না হলেও এটা বৃহত্তর স্বার্থে কাজে লাগবে। আমি নিচের বিষয়গুলো নিয়ে সন্তুষ্ট আছি।

১) যেকোনো সময় এই গবেষণা থেকে নিজেকে সরিয়ে নেয়ার অধিকার আছে আমার।

২) আমার যে তথ্য সংগ্রহ করা হবে টা গোপন থাকবে এবং শুধুমাত্র গবেষণার কাজে ব্যবহার করা হবে।

৩) কোন সমস্যা বা প্রশ্ন থাকলে আমি যেকোনো সময় গবেষকের সাথে যোগাযোগ করতে পারব।

আমি তথ্যগুলো পড়েছি, বুঝেছি এবং এই গবেষণায় অংশগ্রহণের জন্য রাজি আছি।

অংশগ্রহণকারীর নামঃ গবেষকের নামঃ

স্বাক্ষরঃ স্বাক্ষরঃ

তারিখঃ তারিখঃ

Appendix C: Questionnaire

Quantification & scaling of risk factors of diabetes mellitus and isolation of microorganism from diabetic foot ulcer: a cross sectional study.

Particulars of the participant

Id	Date of data collection:
Name of participant:	Age:
Sex:	Occupation:
Marital status: ☐ unmarried ☐ Married ☐ Widow ☐ Divorced	Address:
Educational status: ☐ Illiterate ☐ Primary ☐ SSC ☐ HSC ☐ Graduation ☐ Postgraduation	Socioeconomic status: ☐ Lower class ☐ Middle class ☐ Upper class
Weight: kg	Height: cm
Type of DM: Type 1 Type 2	Duration of DM: months
Name of Hospital:	Admission Date:
Mobile number:	

A. Blood sugar level measurement:

1. Fasting blood sugar .....mg/dl
2. Random blood sugar.....mg/dl
3. HbA1c .....%

B. Grading of foot ulcer: Please put tick mark on right sided empty box

Grade 0- Intact skin	
Grade 1- superficial ulcer of skin or subcutaneous tissue	
Grade 2-ulcers extend into tendon, bone, or capsule	
Grade 3-deep ulcer with osteomyelitis, or abscess	
Grade 4-partial foot gangrene	
Grade 5-whole foot gangrene	

C. Other information from ulcer:

4. Size of the ulcer..... mm

5. For how long the ulcer was found..... Days

6. Organism found in Ulcer : ☐ *E. Coli* ☐ *Staphylococcus* ☐ *Pseudomonas* ☐ Others

D. Previous History:

7. Previous history of foot ulcer: ☐ Yes ☐ No

   If Yes, any previous history of antibiotic taken ☐ Yes ☐ No

   If Yes, which Antibiotic ☐ Ceftriaxone ☐ Flucloxacillin ☐ Amikacin ☐ Meropenem ☐ Others

   For how long Antibiotic ……..days.

8. Previous history of gestational diabetes mellitus (in case of Female patient)

☐ Yes ☐ No

E. Medication information:

9. What type of medication is taking?	Starting date
☐ Oral Hypoglycemic agents	
☐ Insulin	

F. Risk factor of Diabetes Mellitus: Please put tick mark on box on following table

10.Family history of DM	☐ Among relatives ☐ Among siblings ☐ Among one parent ☐ Both parents ☐ At least 1 siblings & parents
11.BMI	☐ <18.5 ☐ 18.5-24.9 ☐ 25-29.9 ☐ >30
12.Life style	☐ Regular moderate physical activity ☐ Irregular moderate physical activity ☐ Mild physical activity ☐ sedentary life style (neither exercise nor walk). Harmful personal habits: • Smoking history ☐ Yes ☐ No • Alcohol history ☐ Yes ☐ No • Betel leaf consumer ☐ Yes ☐ No

13.Level of awareness	☐ Appropriate knowledge & practice ☐ Moderate knowledge & moderate practice ☐ Partial knowledge & irregular practice ☐ Partial knowledge & occasional practice ☐ No knowledge & no practice

14. Diet history:

Foods having daily : ➢ Rice ➢ Ruti ➢ Sugar	Times: ☐ 1 ☐ 2 ☐ 3 ☐ 1 ☐ 2 ☐ 3 …….teaspoon
Foods having weekly: ➢ Meat ➢ Fish ➢ Egg ➢ Vegetables ➢ Fruits	 …….times …….times …….times …….times …….times

G. Co-morbidities present:

15.Hypertension	Yes	No	If yes, for how long? (in months)
16.Cardiovascular diseases			
17.Cerebrovascular diseases			
18.Dyslipidemia			

H. Complications are suffering by participant:

Complications	Yes	No	If yes, Duration (in months)
19.Retinopathy			
20.Nephropathy			
21.Neuropathy			
22.Amputation			
23.Vascular diseases			

Signature and date of data collectors:

Brief biography

Rinky Sharma passed the Secondary School Certificate Examination in 2008 followed by Higher Secondary Certificate Examination in 2010. I have obtained my MBBS 2017 from Institute of Applied Health Science, USTC, Chattogram. Bangladesh. Now, I am a Candidate for Thesis defense, One health Institute, Faculty of Veterinary Medicine, CVASU. I have passed MRCP (Membership of Royal College of Physicians UK) part 1 exam on January 2020. I am currently working as a Medical doctor in COVID-19 Response in International Rescue Committee (IRC) Bangladesh since June 2020. I have immense interest to work with diabetic foot ulcer patients in mass spectrometry based antibiotic residues in microorganisms.