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Bacterial profile and Antimicrobial susceptibility pattern of isolates from diabetic foot infections at selected public Hospitals of Addis Ababa, Ethiopia.

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This is to certify that the thesis prepared by Bereketeab Berhanu, entitled:

"Bacterial profile and Antimicrobial susceptibility pattern of isolates from diabetic foot infections at selected public Hospitals of Addis Ababa, Ethiopia." and submitted in partial fulfillment of the requirements for Master of Science degree in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology) complies with the regulations of the University and meets the accepted standards with respect to originality and quality.

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List of abbreviations

AAU	Addis Ababa University
ALERT	All African Leprosy, Tuberculosis and Rehabilitation Training Center
AST	Antimicrobial Susceptibility Testing
ATCC	American Type Culture Collection
BMI	Body mass Index
CLSI	Clinical and Laboratory Standard Institute
CoNS	Coagulase Negative Staphylococcus
CRE	Carbapenem resistant Enterobacteriaceae
DFI	Diabetic Foot Infection
DFU	Diabetic Foot Ulcer
ESBL	Extended spectrum beta lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
IWGDF	International Working Group on the Diabetic Foot
TSI	Triple Sugar Iron
MDRO	Multi drug resistant organisms
MRSA	Methicillin resistant *Staphylococcus aureus*
MSSA	Methicillin Sensitive *Staphylococcus aureus*
NCCLS	National committee for clinical laboratory standards
PVD	Peripheral vascular disease
SPSS	Statistical Package for Social Sciences
TASH	Tikur Anbessa Specialized University Hospital
UK	United Kingdom
VRE	Vancomycin resistant enterococcus
WHO	World Health Organization

Abstract

Background: Globally, diabetic foot infections continue to be a major public health problem, bringing socio economic burdens to the affected people. Clinically infected foot ulcers require treatment guided by appropriate cultures and antimicrobial susceptibility testing. Updated information is scarce in Ethiopian context in general and in the study sites in particular, hence we tried to fill this gap.

Objective: To determine the Bacterial profile and Antimicrobial susceptibility pattern of isolates from Diabetic foot infections at selected public Hospitals of Addis Ababa, Ethiopia.

Method: A cross sectional design was used to recruit 135 diabetic adult patients with diabetic foot infections attending selected public hospitals in Addis Ababa, Ethiopia. Convenient sampling was employed in recruiting participants for one year from May, 2018 to April, 2019. Wound Aspirates (deep wound swabs) from the foot ulcers were collected aseptically and inoculated into Blood, MacConkey, Chocolate and Manitol salt Agar. The antimicrobial susceptibility patterns were conducted according to the criteria of the National Committee for Clinical Laboratory Standards (NCCLS) by disk diffusion method. A structured questionnaire was used to collect information regarding the socio-demographic status, clinical history and risk factors of the study participants. Data obtained was analyzed using the statistical package for social sciences software version 20. Statistical significance was set at 95% confidence level and p values ≤ 0.05 was considered significant. The associated factors of DFI were determined using multiple ordinal regressions with the test of parallel line assumption being fulfilled for each risk category separately.

Results: Of the 135 patients investigated majority105 (77.8%) of them were males. The Mean age (SD) of the patients was 57.64 (± 13.20 SD) Years. According to the International Working Group on the Diabetic Foot (IWGDF) classifications, mild, moderate and severe Diabetic foot infections in our study were 36 (26.7%), 75 (55.5%) and 24(17.8%), respectively. One hundred ninty bacterial isolates were identified among 135 patients. Among them, 85 (62.96%) had mono bacterial infection while 50 (37.04%) had mixed bacterial infections. Gram negative aerobic bacterial infections were more accounting 121 cases (63.7%) than gram positive aerobic bacteria 69, (36.3%). The most commonly isolated bacteria was *S. aureus* (26.3%), followed

by *Klebsiella spp* (22.1%) and *Proteus spp* (11.1%). In general, 140(73.68%) of the isolates in our study developed multidrug resistance to at least one drug in three different classes of antibiotics. Meropinem and Amikacin appeared to be the best antibiotics for therapy against Gram negative and Cefoxitin and Vancomycin against gram positive organisms. Moreover, Health education on proper foot care (AOR=3.743, 95% CI 1.615-8.674), Peripheral Vascular Disease (AOR=0.298,95% CI 0.116-0.765), Nephropathy (AOR=0.354, 95% CI 0.135-0.927) ,BMI normal (AOR=0.052,95% CI 0.004-0.663) and overweight (AOR=0.072, 95% CI 0.006-0.935) were found to be associated with Severity of Diabetic foot infection.

Conclusion: High level of multidrug resistance in this study implies, definitive therapy should be based upon culture and susceptibility analysis to promote the rational use of the Antibiotics and reduce emergence of bacterial resistance to antimicrobials.

Key words: Diabetic foot infection, Mild, Moderate, Severe, Poly microbial, Mono microbial

1. Introduction

1.1 Background

In the past 20 years the prevalence of diabetes among the world's adult population has raised by morethan three fold, growing to over 463 million adults World Wide. During this time global prevalence of Diabetes mellitus has dramatically increased from 4.6% to 9.3% [1].As the prevalence of diabetes mellitus increased, complications associated with the condition also has increased dramatically in recent decades [2].

Diabetic foot infection (DFI) is one of the devastating complications characterized by local findings of inflammation or purulence (sometimes accompanied by systemic manifestations of sepsis) occurring in a site below the malleoli in a person with diabetes [3]. In addition to inflicting intense morbidities, DFIs now account for the highest number of diabetes related hospital bed days and the most common proximate, non traumatic cause of amputations [3,4].

Major risk factors are peripheral neuropathy, peripheral arterial disease, and impaired immunity[5].More than one half of non traumatic lower extremity amputations are related to diabetic foot infections, and 85% of all lower extremity amputations in patients with diabetes are preceded through an ulcer [6,7].

A review of the epidemiology of diabetic foot problems in Africa highlighted not only the frequency of neuropathy, however the increasing frequency of peripheral vascular disease, presumably an end result of growing urbanization and further elements like: unhygienic conditions, poverty, inappropriate foot wear, barefoot gait, low income, and cultural practices had been additionally glaring as a risk factor [8].

Diabetic foot ulcer (DFU) infection is classified as mild, moderate or severe according to the extent and severity of the clinical signs, and whether systemic symptoms are present [9]. Osteomyelitis may also additionally arise in any of those categories [10]. The diagnosis of infection in a DFU is made largely on a clinical basis. However, if infection is suspected, the DFU ought to be sampled for microbiological analysis [11, 12].

Foot infection is one of the most common bacterial infections in clinical practice of diabetes.Many research stated at the bacteriology of diabetic foot infections (DFIs) over the last 25 years, however the results have variations and have often been contradictory. The difference could partly have been due to the variations in the causative organisms, over time, geography, or the type and the severity of the infection, as were reported in the studies [13]. Diabetic foot infections are predominantly poly microbial [14]. A combination of gram-positive and gram-negative aerobes (e.g., *Escherichia coli*, *Proteus* species, and *Klebsiella* species) with anaerobes is likely to be found at the site of infection [15]. For patients who haven't been treated with antibiotics within the past thirty days and have a mild DFI, infections are often mono microbial. The most common causative organisms are aerobic gram positive bacteria present on the skin surface such as β hemolytic streptococci or *Staphylococcus aureus*. Methicillin-resistant *Staphylococcus aureus* is present in 10% to 32% of diabetic infections and is associated with a higher rate of treatment failure in patients with diabetic foot infection [16]. In contrast, infections are typically poly microbial in patients with diabetes who have used antibiotics within the past thirty days and in people with deep, limb threatening infections or persistent non healing wounds. Anaerobic bacteria are generally part of poly microbial infections in wounds with malodorous discharge, limb ischemia, or gangrene. [13].

Bacteriological assessment of diabetic foot ulcer infection is essential to identify those agents that are involved in the development of the foot lesions. Knowledge of the bacteriology of diabetic foot infections is significant in guiding antibiotic selection and appropriate definitive therapy that will help health care professionals to manage diabetic patients and prevent from subsequent amputation [17]. Antibiotic susceptibility test is also a requirement for the management of infections which can help to make better therapeutic choices. Hence, this study was aimed to determine the organisms associated with diabetic foot infection (DFI) and their antibiotic sensitivity pattern in selected public hospitals of Addis Ababa.

1.2. Statement of the problem

Globally, diabetic foot ulcer infections are one of the major public health problems leading to socioeconomic burden to the suffering individuals [18, 19]. About half of all DFUs are clinically infected at the time of presentation .And such an infection is a major threat in DFUs, more so than in wounds of other etiologies not subject to diabetic changes. It has potentially serious implications because of its ability to destroy tissue, and its association with amputation [20, 21, 22].

Diabetic foot infection increases the risk of undergoing a lower extremity amputation by 15 fold compared to those without diabetes [23]. Twenty percent of all diabetic persons enter hospitals from foot problems. Almost half of diabetic patients' hospital bed occupancy is due to foot problems. Apart from the morbidity and mortality associated with diabetic foot ulcers and amputations, the economic and emotional impact for the patient and the family can be enormous. [24] In Ethiopia DFI accounted for 21.0% inpatient mortality and sepsis was the most idenified cause in nearly 60.0% of these cases [25].

Currently another emerging problem for both developed and developing countries is increase in both community and hospital-acquired antimicrobial resistant bacteria. This increased rate of isolation of antibiotic resistant pathogens, particularly methicillin resistant *S. aureus* (MRSA), vancomycin resistant *enterococci* (VRE), extended-spectrum β-lactamase- (ESBL) or carbapenamase producing gram negative bacilli and highly resistant strains of *P. aeruginosa* has been the major problem in treating DFIs in the past few decades. The potential presence of such resistant isolates emphasizes the importance of obtaining optimal specimens for culture and sensitivity testing for infected DFIs [6, 26].

Since many different organisms, alone or in combination, can cause DFI, choosing the most appropriate antibiotic therapy requires defining the specific causative pathogens [6, 18, 27]. Empirical antimicrobial therapy is often initiated based on susceptibility data extrapolated from studies performed on general clinical isolates. However, in Ethiopia there are only two related studies few years back [25,28] that revealed an overall prevalence of infection in diabetic patients and indicated that Diabetic foot infection was the leading accounting for 35% of the

infections [28]. Both studies lack the antimicrobial susceptibility pattern data and also one of the studies was conducted from secondary data. Hence the principal objective of this study was to determine the bacterial profile of infected diabetic foot ulcers and the current antibiotic resistance pattern of the bacterial isolates in selected public hospitals in Addis Ababa, Ethiopia. And this will fill the gap we have described above.

1.3. Significance of the study

There is scarce information in Ethiopia on isolation and Antibiotic susceptibility patterns of bacteria from Diabetic Foot infections. The findings of this study will be beneficial for future determinations of empirical therapy policies for the management of DFIs.

The increasing rate of drug resistance in different organisms will show such studies are required for the identification of organisms and the drug which is effective for that specific isolates. For that reason this study will assist clinicians to prescribe the appropriate antibiotics and helps the patients in getting timely and appropriate treatment.

In addition to this, identification of associated risk factors of DFI in our study is helpful in guiding diagnosis, therapy or disease control.

2. Literature review

2.1. Disease Burden

Diabetes and its complications are rapidly becoming the leading cause of global morbidity and mortality. It is predicted that by 2045, there will be about 700 million people with diabetes worldwide [29]. A person with diabetes has a lifetime risk of about 25% of developing a foot ulcer and a lower limb is lost of diabetes every 30 seconds somewhere in our world [30].

2.2. Bacterial Profile of Diabetic foot Infection

In a revew of the literatures on DFIs published in the past 30 years, starting in the late 1970s, I. Uckay et al, demonstrated Gram positive aerobic cocci (especially *Staphylococcus aureus*) as the predominant pathogens commonly refered as a mono microbial infection and aerobic gram negative bacilli were mainly seen in patients with chronic diseases who have received treatment. In recent years DFIs caused by multidrug resistant organisms(MDRO), such as a gram negative bacillus that produces extended spectrum β lactamase or methicillin resistant *S. aureus* (MRSA), have emerged as a major problem. On the other hand investigations in warm climates (especially India, but also the Middle East and Africa) showed that the most common isolates were Gram negative bacilli, especially *Pseudomonas aeruginosa*.This literature review of DFI studies revealed that anaerobes were infrequent isolates having no association with any specific clinical findings and not clearly leading to more severe manifestations. *Bacteroides and Peptostreptococcus* were the most commonly reported species [31].

Diabetic foot infections can either be mono microbial or poly microbial. Citron et al, in a study done as part of a United States based multi center clinical trial to determine the bacteriology of moderate to severe diabetic foot infections, found that 83.8% of DFU infections were poly-microbial, 43% only had aerobes, 43.7% had both aerobes and anaerobes and 1.3% had only anaerobes. Oxacillin susceptible *Staphylococcus aureus* were the majority aerobic organisms. The predominant anaerobes were gram positive cocci, *Prevotella* species, *Porphyromonas*

species and the *Bacteroides fragilis* group. The study concluded that moderate to severe DFU infections were typically poly-microbial and almost half comprised of anaerobes [13].

In a prospective study at the Federal University of Ceará, Brazil, 298 species of bacteria isolated from 141 patients with community-acquired diabetic foot ulcers were analyzed. They used conventional and automatic identification and sensitivity testing techniques. The most common isolates were *Enterobacteriaceae* (83.7%), *Staphylococcus aureus* (43.3%) and anaerobic bacteria (17%). *Streptococcus pyogenes* was recovered from 7.8% of the cases. ESBL producing strains were detected in 6% and methicillin resistant *Staphylococcus aureus* strains were recovered from 11.6% of the patients [32].

In a Clinical and bacteriological study on diabetic foot infections in Lisbon, Portugal, Forty nine inpatients and outpatients, were recruited and cultured 147 strains of microorganisms.Staphylococcus was the main genus identified, and methicillin resistant *Staphylococcus aureus* (MRSA) was present in 24.5% of total cases. Of the clinical samples collected from patients receiving antibiotics, 93% of the antibiotic regimens were considered inadequate based on the antibiotic susceptibility test results. The median ulcer duration of each isolated multi drug resistant (MDR) microorganism was 29 days, and previous treatment with fluoroquinolones was statistically associated with multi-drug resistance [33].

In a Turkish study, Turhan et al, isolated more gram negative organisms indicating an increase in their incidence in DFUs with 83.5% of the patients showing mono bacterial and 16.4% poly microbial infections. Gram negative bacteria were more isolated at 61.3% compared to gram positive ones at 38.7%. Out of the gram negatives, 13.2% were beta lactamase producers while methicillin resistant *S. aureus* isolates were 44.2%. According to the study, most gram negative organisms were sensitive to vancomycin. Fusidic acid was active against all strains of *Staphylococcus* including the methicillin-resistant ones. Majority of the gram negative isolates were sensitive to sulbactam/cefoperazone and tazobactam/piperacillin with most bacteria isolated sensitive to ceftazidime, amikacin and imipenem [34].

In a prospective hospital based study of diabetic foot patients in India for a year and six months 87 organisms were isolated from70 specimens, including *Escherichia coli* (19.5%) among the Gram negative and *Staphylococcus aureus* (18.4%) among the Gram positive as the predominant

aerobes explored. The study indicated that *Pseudomonas aeruginosa* and *E. coli* were predominant isolates of non healing ulcers. The antimicrobial sensitivity pattern revealed that vancomycin (100%) and amikacin (90.4%) exhibited highest sensitivity to Gram positive cocci, while all strains of *P. aeruginosa* were sensitive toward imipenem (100%) [35].

A cross sectional study conducted in Nemazee Hospital, Shiraz, Iran for a period of 24 months from 2012 to 2014 for isolation and Antimicrobial susceptibility of isolates from DFI isolated 122 aerobic microorganisms. Among Gram positive and Gram negative bacteria, *Staphylococcus spp*. and *E. coli* were organisms isolated predomiantly, respectively. And the study indicated that 91% of the isolates were multi drug resistant while MRSA accounting about 78% of *S. aureus* isolates.And 53% of Gram negative bacteria were positive for extended spectrum β-lactamase [36].

A retrospective study conducted in a university hospital in Kuwait from June to July 2007 with the aim of determining the microbiological profile of diabetic foot infections (DFIs) and assessing the antibiotic susceptibility of the causative agents showed that most of the isolates were Gram negative pathogens accounting for 51.2% and the Gram positive pathogens (32.3%) or anaerobes (15.3%). Polymicrobial infections were found in 75% of patients. The main isolated organisms were members of the *Enterobacteriaceae* family (28.5%), Pseudomonas *aeruginosa* (17.4%), Staphylococcus *aureus* (11.8%), methicillin resistant S. *aureus* (7.7%), Gram negative anaerobes (10.8%) and *Enterococcus* spp. (7%). Vancomycin is the most effective method for treating Gram positive bacteria and imipenem, piperacillin tazobactam and amikacin were the most effective treatments for the Gram negative bacteria [37].

A Review of the bacteriological results of 111 specimens of patients with continuous diabetic foot infections from King Abdulaziz, Jeddah, Kingdom of Saudi Arabia, during the period January 1997 to June 1999 indicated that *Staphylococcus aureus* having been the most common strain was detected in 28% of cases, including 9 out of 30 (30%) patients with methicillin-resistant Staphylococcus aureus. Other isolated organisms are Pseudomonas aeruginosa (22%) and Proteus mirabilis (18%), Gram-negative anaerobic organisms (11%), mainly Bacteroides fragilis.The antimicrobial susceptibility testing, showed that vancomycin was the most effective

against gram-positive and imipenem was the most effective against gram negative organisms [38].

A study was conducted to determine the bacterial profiles of DFI and the antibiotic resistance pattern of the isolates at the Yaoundé Central Hospital in Cameroon. One hundred forty eight bacterial isolates were obtained from 56 positive cultures, and each case contained an average of 2.5 microorganisms. Gram negative bacilli were the predominant isolates accounting about 65.5%. Monomicrobial growth was observed in 18.59% and sterile growth in 6.25% of the isolates. The most common isolates among Gram negative bacteria were Proteus (21.6%), Escherichia coli (18.9%), Klebsiella Species (16.9%) and *Pseudomonas aeruginosa* (8.1%). Among the Gram positive organisms Staphylococcus aureus was predominant (17.6%), followed by *Staphylococcus epidermidis* (10.1%) and *Streptococcus pyogenes* (6.8%). The Antimicrobial susceptibility results showed that Gram negative bacterial isolates were 100% sensitive to Imipenem and 86.5% resistance to Ampicillin, while for the Gram positive bacterial, they were 44.5% sensitive to Ciprofloxacin and 46.8% resistance to Oxacillin [39].

The bacteriology of diabetic foot infections studied in Egypt found that most patients had mono bacterial foot infections and that gram negative bacteria (67%) were most commonly isolated compared with gram positive bacteria (30%). The most commonly isolated gram positive organisms were *S. aureus* (10.2%), *S. pyogenes* (7.1%) and methicillin resistant *S. aureus*. The predominant gram negative organisms were *P. aeruginosa* (19.4%), *K. pneumonia* (15.3%) and *Acinetobacter species* (10.2%). The gram positive bacteria were most susceptible to vancomycin and imipenem. Amikacin was the most effective agent against gram negative bacteria [40].

A retrospective study conducted among an Egyptian population by El-Sheikh et al isolated S. aureus 41.9% followed by Coagulase-Negative *Staphylococci* (CoNS) 9.7%, *E. coli* (9.7%), *Klebsiella* (9.7%) and *P. aeruginosa* (6.4%). *Staphylococcus aureus* was sensitive to cephalexin, dicloxacillin, amoxicillin-clavulanate and trimethoprim-sulfamethoxazole. Pseudomonas aeruginosa showed sensitivity to carbenecillin and levofloxacin. Escherichia coli were sensitive to amikacin, imipenem, piperacillin and ceftazidime [41].

A prospective study on the microbiological causes of DFI with special focus on their antimicrobial resistance pattern among diabetic patients treated at Cairo University hospitals was conducted by Dwedar et al. According to the study, gram negative bacteria were isolated more at 56.08% compared to gram positive organisms at 27.7%. Gram negative anaerobes and Candida species accounted for 8.1% each. The predominant organism isolated was *P. mirabilis* (16.8%), then *E. coli* (13.5%), Methicillin Sensitive *Staphylococcus* (MSSA) (11.4%), *Pseudomonas species* (10.8%) and MRSA (10.1%). The study indicated that vancomycin was the most effective drug against gram-positive bacteria and the most effective drugs against gram-negative bacteria were imipenem, amikacin and colistin [42].

A cohort prospective study conducted by Widatalla AH et al. in Jabir AbuEliz Diabetic Center (JADC), Khartoum, Sudan including 330 diabetic patients with foot osteomyelitis (study group) and 1,808 diabetic patients with a foot ulcer but without foot osteomyelitis (control group) indicated that *Staphylococcus aureus* was the most common pathogen isolated in diabetic foot osteomyelitis, followed by other aerobic Gram-positive cocci. Aerobic Gram-negative bacilli and anaerobes were occasionally isolated, often in mixed diabetic foot infections. But a higher than usual incidence of *Pseudomonas aeruginosa* osteomyelitis was noticed [43].

Feleke Y et al, conducted a cross sectional study to assess the prevalence and pattern of infection, determine the causative organisms among 176 Diabetic patients admitted to the department of internal medicine of Tikur Anbessa Specialized University Hospital between Nov. 2000 - Nov 2002. A total of 114 clinical specimens (40 pus, 23 blood, and 51 urine) were sent to the microbiology laboratory for bacterial isolation and identification. The study revealed an overall prevalence of infection to be 44% in diabetic patients. The most common infection in diabetic patients was diabetic foot infection (35%), followed by tuberculosis (22%), urinary tract infection (14%) , pneumonia (12%) and skin and subcutaneous infection (12.8%). About 30% of the bacterial isolates were *Staphylococcus aureus* followed by *Klebsiella pneumonia*(23.4%), *Escherchia coli*(19%) and *Pseudomonas spp*(15%). The remaining isolates are rare, between 2% and 6.4%.More than one bacterial species were also isolated from 10% of the positive culture [28].

Similarly a retrospective study was done to determine the various risks as well as antecedent factors, other long term complications, treatment profile and subsequent follow up of 196 patients with diabetic foot disease admitted to the Tikur Anbessa Specialized Referral Hospital from Jan 1999 to Dec 2003. Bacteriological culture study from the wound site or discharge was made for aerobic organisms in 119 (61.0%) of the cases. Sixty three (53.0%) had growth for a single organism, *Klebsiella species* being the commonest isolate and 31 (26.0%) were polymicrobial. 27 (23.0%) of the media had no growth [25].

2.3. Risk Factors of Diabetic Foot Infections

Even though studies on DFI specifically are scarce in the world, findings from few studies show that different factors were associated with its occurrence. A prospective, multicenter study was conducted to determine risk factors for foot infection in a cohort of people with diabetes. The researchers found that bone contact on probing, foot ulcer duration of longer than 30 days, a history of recurrent foot ulcers, traumatic etiology of the ulcer and peripheral vascular disease were the major factors significantly associated with DFI [44].

Another retrospective review of diabetic lower extremity infection and the influence of physical, psychological, and social factors found that risk factors for severe diabetic foot infection were previous amputation, peripheral vascular disease and neuropathy [45].

Other prospective follow up analysis of a Eurodiale subgroup study have identified walking barefoot as a risk for infection. Of note, these risk variables are similarly associated with recurrence of ulcers [46] or reinfection after successful treatment.

However, data on the incidence and influencing factors of foot ulcer infections in Ethiopia are limited. So emphasis is required to conduct this type of studies a lot.

2.4. Conceptual framework

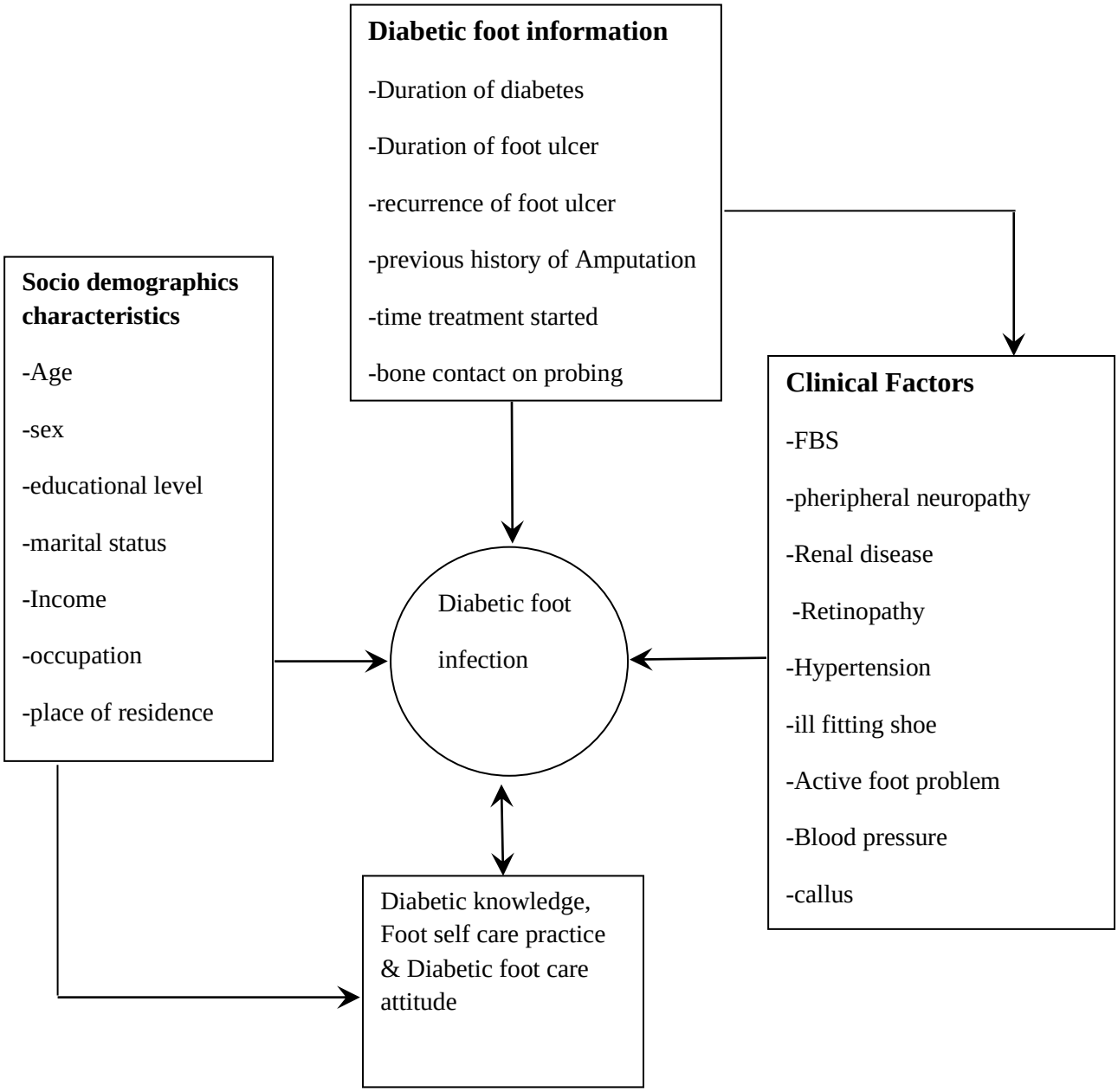

Figure 1. Conceptual framework of possible factors associated with Diabetic foot infection

Various studies related to diabetes [44, 45, 46], diabetic foot ulcer and diabetic foot infection are reviewed to develop this conceptual frame work. Variables in each box except the box containing diabetic foot infection are associated risk factors. The double arrow indicates the reverse effect of the one variable on the other.

3. Objective of the study

3.1 General Objective

To determine the Bacterial profile and Antimicrobial susceptibility pattern of isolates from DFI at selected public hospitals in Addis Ababa, Ethiopia from May,2018 to April, 2019.

3.2 Specific Objectives

1. To identify the bacterial isolates from diabetic foot ulcer specimens from adult diabetic patients at TASH, ALERT, St. Paul, Yekatit 12, Zewditu, Ras Desta and Minilik Memorial Hospitals.

2. To determine drug susceptibility pattern of isolates from DFIs at selected public Hospitals in Addis Ababa, Ethiopia.

3. To identify factors associated with DFIs at selected public hospitals in Addis Ababa, Ethiopia.

4. Hypothesis

4.1. Null Hypothesis

The Bacterial profile of Diabetic Foot infections at selected public hospitals in Addis Ababa is the same with previous studies conducted in Ethiopia.

5. Materials and Method

5.1. Study Area

The study was conducted in patients with only Diabetic foot infections attending the hospitals (TASH, St. Paul's, ALERT ,Yekatit 12, Zewditu, Ras Desta and Minilik Memorial Hospitals) in Addis Ababa, Ethiopia. The study was conducted in the medical, surgical, orthopedic wards and also at the diabetes outpatient clinics. TASH is the largest referral hospital in the country, with 700 beds which was transferred to school by the federal ministry of Health in 1998, and it has since become a university teaching Hospital. Currently in its Diabetic center 60 to 80 diabetic patients are clerked per day and every Friday 5 to 8 patients visit the center with foot ulcer case and annually approximately 10, 000 DM cases were registered [47].

Likewise St Paul's hospital is also one of the largest referral hospitals in the country with 350 beds seeing an annual average of 300,000 patients. It also do have similar no of registered diabetic patients to TASH. ALERT is another hospital which focuses on its hospital, rehabilitation of leprosy patients, training programs for leprosy control. Currently with its 240 bed teaching hospital giving dermatology, ophthalmology, and Surgery departments, also orthopedic workshop, and a Rehabilitation program.

Minilik II memorial hospital is also another Central referral hospital giving different medical services for Diabetic patients. Currently on average 30 to 40 patients visit this department every week. The other hospital the study will be carried out is Yekatit 12 Hospital. Which is also located at Tewodros street in front of Sidist kilo square Addis Ababa. It is also a Medical college. According to their HMIS registration, The Diabetes Center provides comprehensive diabetes care to around 1500 diabetic patients per year. Ras Desta and Zewditu are memorial hospitals found in Addis Ababa under the administration of Addis Ababa city Health Bureau.They also give different medical services for large number of diabetic patients in the city.

5.2. Study design and Study period

A cross sectional study was conducted from May 01, 2018 to April 30, 2019 at TASH, ALERT, St. Paul's, Yekatit 12, Zewditu, Ras Desta and Minilik Memorial Hospitals.

5.3. Population

5.3.1. Source population

All diabetic patients who visited TASH, ALERT, St. Paul's, Yekatit 12,Zewditu, Ras Desta and Minilik Memorial Hospitals during the study period.

5.3.2. Study population

All Diabetic patients with Foot ulcer who visited TASH, ALERT ,St. Paul's, Yekatit 12,Zewditu,Ras Desta and Minilik Memorial Hospitals during the study period that fulfilled the eligibility criteria.

5.4. Inclusion and Exclusion criteria

5.4.1. Inclusion criteria

- Diabetic Patients with Foot infection
- Patients have to be above 18 years and agree to participate in the study and give informed consent.

5.4.2. Exclusion criteria

- Patients who were on antibiotic treatment within 14 days of data collection**.**

5.5. Study variables

5.5.1. Dependent variables

- Antimicrobial susceptibility pattern
- Bacterial isolates
- Severity of DFI

5.5.2. Independent variables

- Sociodemographic variables: age, sex, marital status, educational background,occupation, area of residence, and average monthly income

- Behavioural factors: smoking cigarette, alcohol consumption, and physical activity
- Clinical factors: Active foot problem, comorbidity (additional known disease), body mass index, regular follow up to the diabetic clinic, category of diabetes, peripheral vascular disease, neuropathy, Retinopathy,Nephropathy,proper health education and duration of diabetes mellitus
- Foot selfcare practice related factors: characteristics of foot wear, footwear inspection, footwear practice, and foot washing.

5.6. Measurement and Data collection

5.6.1 Sample size calculation and sampling method

5.6.1.1. Sample size determination

The sample size was calculated based on single sample size estimation. The value of p taken as 9.7% (0.097) from the previous study conducted on Trend of diabetic admissions in Tikur Anbessa and St. Paul's University Teaching Hospitals, Addis Ababa Ethiopia [48]. Considering 95% confidence interval, 5% margin of error and 9.7 proportions, the sample size is calculated using the following standard formula.

$N = (Z \alpha/2)2 * (1-p) * (p)/(d)2$

Where n=sample size estimated

α = level of significance

z = at 95% confidence interval Z value ($\alpha = 0.05$) =>$Z \alpha/2=1.96$

d= Expected margin of error =0.05

p=prevalence of previous study found from literature review= 9.7% [48]

$n = (1.96)2 *0.097(1-0.097)/(0.05)2$

n=135

5.6.1.2. Sampling Technique

The public hospitals were selected using a non-probability convenient method from total public Hospitals in Addis Ababa. A consecutive sampling technique was used to enroll the study participants. All consecutive Diabetic patients who came to TASH, ALERT, St. Paul's, Yekatit 12, Zewditu, Ras Desta and Minilik Memorial Hospitals medical, surgical, orthopedic wards and also at the diabetes outpatient clinics with foot infection were included. A total of 135 informed and consented diabetic patients with foot ulcers were investigated for bacterial infection. Of these, 47 were from TASH, 21 from St. Paul's, 38 from ALERT, 9 from Minilik II Memorial, 5 from Yekatit 12, 12 from Ras Desta, and Three from Zewditu hospital. Since DFI is not a very frequent incidence the sample distribution is not organized.

5.6.1.3. Selection and evaluation of study subjects

Convenient sampling technique was applied to select the study subjects. Therefore, the doctors in each unit conducted a thorough clinical examination of the patients.All Patients with Diabetic foot infection that fulfilled the eligibility criteria during the study period were selected.

5.6.2. Data collection procedures

Permission to carry out the study was sought from the Medical directors of the Hospitals, laboratory and the consultants in charge of the diabetes outpatient clinic or the wards at all the seven hospitals. Primarily Physicians in the diabetic clinic and the wards were communicated for their collaboration in the sampling of the wound aspirates or deep wound swab. And a data collection activity was performed with the assistant researchers and a laboratory technologist helped in the laboratory bench work activities. Structured and Predesigned questionnaire was developed and used for collection of data on socio demographic characteristics (age, sex, occupation and educational back ground of patients), clinical information and risk factors associated with DFIs. Information that was not obtained through the patient interviews was checked on each participant`s medical record. Information like antimicrobials prescribed for diabetic foot ulcers, grading of the foot ulcer and different laboratory results to confirm the information obtained via the research questionnaire. Isolation, identification and the antimicrobial sensitivity patterns were done in TASH microbiology laboratory. All data were kept under lock and key, with accessibility limited to the researcher only.

5.6.3. Sample collection, handling and transport

Deep wound swabs were aseptically obtained after the wound immediate surface exudates and Contaminants were cleaned with wet sterile gauze and sterile saline solution. Dressed wounds were cleansed off with sterile normal saline after removing the dressing. The specimen was collected by Levine technique on sterile cotton swab by rotating with sufficient pressure. Double wound swabs were taken from each wound at a point in time to reduce the chance of contamination. The swabs were dipped in Amies transport media and transported to the bacteriology laboratory.

5.6.4. Isolation and identification

Culture, Gram staining and Biochemical tests were used. Swabs collected from patients were streaked on a blood agar (5% sheep blood) ,Chocolate agar and MaCconkey agar (Oxoid) and Mannitol Salt Agar (MSA) by sterile inoculating loop. The MacConkey agar plate and MSA were incubated aerobically while chocolate and blood agar were incubated in microaerophilic atmosphere (5-10% CO_2) candle Jar. Preliminary identification of bacteria was done based on colony characteristics of the organisms. Some colony characteristics like haemolysis on blood agar, changes in physical appearance in differential media and enzyme activities of the organisms. Biochemical tests were performed on colonies from pure cultures for identification of the isolates. Gram negative rods were identified by performing a series of biochemical tests-Oxoid using: - Triple Sugar Iron (TSI), Indole test, Simmon's citrate agar, urea, Malonate and motility. Gram-positive cocci were identified based on their Gram reaction, catalase and coagulase test results [49].

5.6.5. Antimicrobial susceptibility testing (AST)

Susceptibility testing was performed by Kirby-Bauer disk diffusion technique [50] according to criteria set by Clinical and Laboratory Standard Institute (CLSI) 2018. The inoculum was prepared from pure culture by picking parts (3-5) of similar test organisms with a sterile wire loop and suspended in sterile normal saline. The density of the inoculation suspension was determined by comparison with the turbidity standard in a 0.5 barium sulfate solution from McFarland. Spreading of the test organisms evenly on the surface of Mueller-Hinton agar (Oxoid) and exposing with the antibiotic impregnated paper disks into the agar medium was

performed, and then incubated aerobically at 37°C for 16–18 hours. Diameters of zone of inhibition around the discs were measured to the nearest millimeter using a clipper and classified as sensitive, intermediate, and resistance according to the standardized table supplied by CLSI 2018. The routine antibiotics that were frequently used in the study area were considered and all the disks that were used for the test are from Oxoid.For gram positive bacteria;- Clindamycin (2µg), Cefoxitin (30µg), Penicillin(10µg), Trimethoprim-Sulphamethoxazole (1.25/23.75µg), Gentamycin (10µg), Tobromycin (10µg), Erythromycin (15µg), Ciprofloxacin (5µg), Ampicillin(10µg), Vancomycin (30µg) , Doxycycline (30 µg) were employed and for gram negative bacteria Tobromycin (10µg), Amoxicilin-Clavulanate(20/10µg), Amikacin(30µg), Gentamycin(10µg), Ampicilin(10µg), Piperacillin-Tazobactam(100/10µg), Cefotaxim (30µg), Cefepime (30µg), Ceftriaxone (30µg), Cefuroxime (30 µg), Chloramphenicol (30 µg), Ceftazidime (30 µg), Ciprofloxacin (5µg), Impenem/ Meropinem (10µg), Trimethoprim-Sulfamethoxazole (1.25/23.75µg) were tested.

5.6.5.1 Detection of Methicillin Resistance

Cefoxitin discs (30 µg) were used for phenotypic testing of MRSA detection. Zone of inhibition equal to or greater than 22 mm was taken as susceptible to Cefoxitin and the organism was reported as Methicillin Sensitive *Staphylococcus aureus*. Those isolates which produced a zone of inhibition less than or equal to 21 mm were considered as Methicillin Resistant *Staphylococcus aureus* (MRSA).

5.6.5.2 Detection of Carbapenem Resistance

A modified Hodge test was used to detect carbapenemase production. A 0.5 Mac Farland's suspension of ATCC Escherichia coli 25922, was diluted 1 in 10 in sterile saline. This was inoculated on a Muellar Hinton agar plate, as for the routine disc diffusion testing. The plate was dried for 5 minutes and a disc of Meropenem 10µg was placed in the centre of the agar plate. colonies of the test organism were picked and inoculated in a straight line, from the edge of the disc, upto a distance of at least 20mm. The plates were incubated at 35 ± 2^0C overnight and they were examined next day. Then were assessed for an enhanced growth around the test organism, at the intersection of the streak and for a zone of inhibition. When there is an enhanced growth

indicated Carbapenemase production, and the absence of an enhanced growth meant that the test isolate did not produce carbapenemase.

5.6.5.3 Detection of Extended spectrum beta-lactamase

The diameter of the zone of inhibition produced by Ceftazidime (30µg), Ceftriaxone (30µg) and Cefotaxime (30µg)was used for initial ESBL screening based on the CLSI screening criteria. These breakpoints indicative of thought for ESBL production are: for CAZ≤22mm, CRO, ≤ 25 mm and for CTX≤ 27mm. Phenotypic detection of ESBL production was confirmed by double disk synergy test and combined disk test according to EUCAST(2018) and CLSI(2018) guidelines respectively.

5.6.5.4 Combined disk (double disk potentiate) Test (CDT)

The phenotypic confirmation of the presence of ESBLs was done by using Ceftazidime (30 µg) disk and Cefotaxime (30µg) disk alone and their combination with Clavulanic acid (30 µg/10 µg). A ≥5 mm increase in zone diameter for either of the Cephalosporin disks and their respective Cephalosporin/Clavulanate disk were interpreted as ESBL producer. This method (according to CLSI) is used as reference phenotypic method for comparing double disk synergy method.

5.6.5.5 Double Disk Synergy Test (DDST)

The organism to be tested was inoculated onto a Mueller Hinton agar plate. The antibiotic disks used are Ceftriaxone (30 µg), Cefotaxime (30 µg), Ceftazidime (30µg), Aztreonam (30µg) and Amoxicillin/ Clavulanic acid (20/10 µg). The four antibiotics were placed at distances of 20 mm (edge to edge) from the Amoxicillin/Clavulanic acid disk placed in the middle of the plate. After 24 hr incubation, if an enhanced zone of inhibition between either of the cephalosporin antibiotics and the Amoxicillin/Clavulanic acid disk occurred, the test was considered positive.

5.6.5.6 Vancomycin resistant Entrococci (VRE)

The disk diffusion test was performed with 6-mm disks with 5 µg vancomycin and Muller Hinton agar plates, using the EUCAST method and clinical breakpoints. According to the EUCAST disc diffusion method, if the diameter of the zone was less than 12

mm, the isolate was classified as resistant. Also, according to the method, resistance should be suspected when the vancomycin zone edge is fuzzy or colonies were growing within the inhibition zone.The isolates were reported as vancomycin susceptible only when the zone edges were sharp and ≥12 mm.

5.7. Data Quality Assurance

The quality of the data collection process was checked by giving adequate training for data collectors & for improvement of the data collection tool corrective measures were taken accordingly for the gap. The questioner was checked for its completeness, readability and clearness using pretest that was delivered for 5% of participants.

Pre Analytical: All specimens were collected based on the standard operating procedures (SOPs). The material was from the actual site of infection. Optimal time of Collection of sample, Appropriate collection devices and specimen containers, Labeling of the specimen, Proper selection of culture media, Specimen transportation, Initial observation and handling of specimen will be performed carefully.

Analytical: Visual inspections of cracks in media or plastic petridishes , unequal fill, hemolysis, evidence of freezing, bubbles, and contamination were performed. The sterility of culture media was checked by incubating 5 % of each batch of the prepared media at 35-37 ºC for 24 hours. Performance of catalase reagent (3% hydrogen peroxide) was checked by known *S. aureus* (positive control) and *S. pyogenes* (negative control). Coagulase test was checked by known *S. aureus* (positive control) and *S. epidermidis* (negative control). For oxidase test *P. aeruginosa* (positive control) and *Escherichia coli* (negative control) were used. Any physical changes like cracks, excess moisture, color, hemolysis, dehydration & contamination were checked before use of all culture medias. Also expiration date were checked strictly. The qualities of all reagents were checked. Temperature of incubator and refrigerator were monitored daily. All prepared medias were checked by inoculating standard strains, such as *Staphylococcus aureus* (ATCC 25923), *Escherichia coli* (ATCC-25922) and *P. aeruginosa* (ATCC-27853) from TASH Microbiology Laboratory as a quality control during study period for culture, Gram stain and antimicrobial susceptibility testing.

Post analytical; It includes reporting of results and analysis of results properly based on the CLSI guidelines. Isolated patient organisms were preserved in Skim Milk Media and stored at -70ºC.And lastly it was ensured that all specimens and cultures were properly handled and finally autoclaved before disposal. Any apparatus used and contaminated were safely disinfected or sterilized.

5.8 Data analysis

The data was entered and double checked before analysis. Then the data was exported to SPSS version 20 for analysis. The descriptive statistics was computed. Binary logistic regression using simple ordinal logistic regression model was done to investigate the association between explanatory variables and outcome variable. Variables which showed significance on the simple ordinal regression model were entered in to Multiple logistic regression.Ordinal regression is a statistical technique that is used here to predict behavior of ordinal level dependent variables with a set of independent variables. The dependent variable is the order response category variable and the independent variable may be categorical or continuous. Parallel lines assumption was met during using the proportional odds model.Statistical significance was set at 95% confidence level and p values ≤0.05 was considered significant. Finally, the result is presented in words, charts, and tables.

5.9. Ethical Consideration

The study was conducted after getting ethical clearance from the Departmental Research and Ethics Review Committee of the Department of Medical Laboratory Sciences,Addis Ababa University and Addis Ababa Health bureau. It was also obtained from Tikur Anbessa Specialized Hospital, ALERT & St Paul's Hospitals. Official permission from Minilik II, Ras Desta, Zewditu memorial and Yekatit 12 hospitals was obtained. Written consent was obtained from each study subjects before collection of swab samples and other relevant clinical information. Study participants did get appropriate treatments based on the findings from the culture and AST. Information obtained at any course of the study was kept in confidential.

5.10. Data presentation and dissemination

The findings of this study on completion could serve as a baseline material for researchers, Clinicians, experts and policy makers for intervention. To reach these bodies the thesis will be Presented and submitted to Addis Ababa University, College of Health Sciences, and Department of Laboratory Sciences. So it can serve as a reference in the library. In addition, a copy of this material were given to TASH, ALERT, St Paul's, Yekatit 12, Ras Desta, Zewditu and Minilik II memorial Hospitals. Additional efforts are also being made to present on conferences to reach the medical/scientific community and publish the article on Peer reviewed Journals after the final reports.

5.11. Operational Definitions

Diabetic Foot Infection: the presence of at least two inflammatory manifestations (purulence or erythema, pain, tenderness, warmth or indurations) [6].

MDRO: - For epidemiologic purposes, MDROs are defined as microorganisms, predominantly bacteria, that show resistance to one or more classes of antimicrobial agents [51].

Mild diabetic foot infection:-Local infection involving only the skin and subcutaneous tissue; if erythema must be 0.5 cm to less than 2 cm around the ulcer (exclude other causes of inflammatory response).

Moderate diabetic foot infection:-Local infection with erythema more than 2 cm around the ulcer or involving structures deeper than skin and subcutaneous tissues, and no systemic inflammatory response signs.

Severe diabetic foot infection: - Local infection with signs of systemic inflammatory response

Multidrug resistance: - Bacteria those are resistant to antimicrobials for a minimal of two or more of classes of antibiotics tested.

6. Work flow

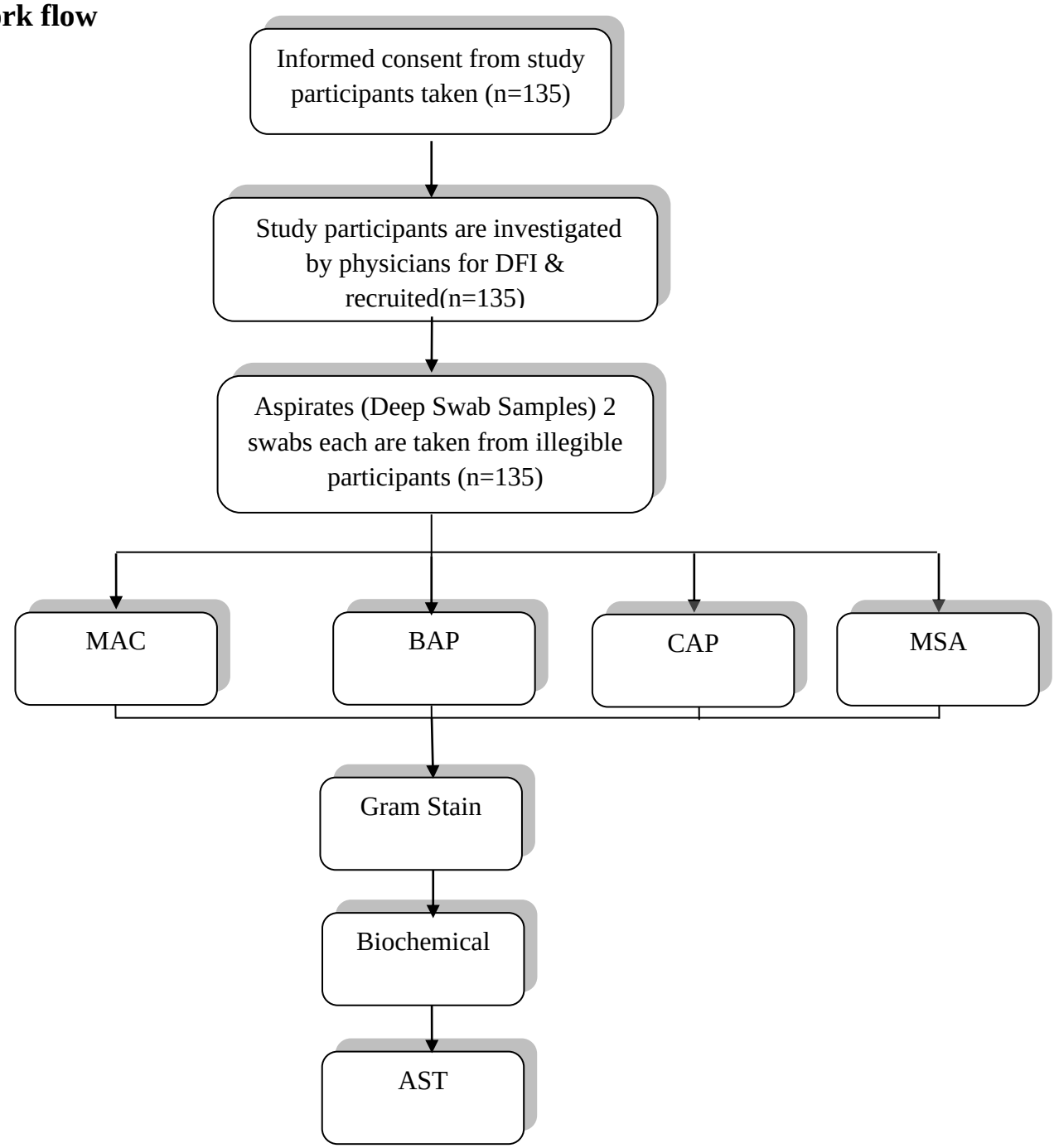

Figure 2. Study Flow Chart for Diabetic patients with foot infections attending selected public hospitals of Addis Ababa, Ethiopia. (May ,2018 –April 2019)

7. Results

7.1. Socio demographic and economic characteristics

A total of 135 study participants were enrolled in this study. Among these, 30 (22.2%) were females and 105 (77.8%) were males. The Mean age (SD) of the patients was 57.64 (± 13.20 SD) Years ranging from 18 to 86 years old. (The age and sex distribution are shown in Figure 3) Majority (85.2%) of the patients were urban residents. Married individuals account for 95 (70.4%) of the patients. One third (33.3%) of the study subjects had college and above education. Fifty five (40.7%) were government or private employee. (Table 1)

Table 1. Socio demographic and economic characteristics of Diabetic foot infected patients at selected public hospitals of Addis Ababa, Ethiopia. (May, 2018 - April, 2019).

		DFI			Total	
Variables	Category	Mild	Moderate	Severe	Frequency	Percent
Age	18-27	1	2	0	3	2.22
	28-37	3	4	1	8	5.93
	38-47	4	11	2	17	12.6
	48-57	10	11	7	28	20.74
	58-67	10	31	9	50	37.03
	>67	8	16	5	29	21.48
Sex	Male	26	60	19	105	77.8
	Female	10	15	5	30	22.2
Residence	Urban	28	67	20	115	85.2
	Rural	8	8	4	20	14.8
Marital status	Single	7	11	5	23	17.0
	Married	24	56	15	95	70.4
	Divorced	2	5	0	7	5.2
	Widowed	3	3	4	10	7.4
Level of education	Illiterate	6	19	7	32	23.7
	Elementary	8	14	3	25	18.5
	High school	10	17	6	33	24.4
	College and above	12	25	8	45	33.3
Occupation	No job/House wife/	7	17	6	30	22.2
	Daily Labor	1	3	0	4	3.0
	Employee	13	33	9	55	40.7
	Merchant	10	15	7	32	23.7
	Farmer	5	7	2	14	10.4
Monthly income	<500	7	16	5	28	20.7
	500-1000	3	7	2	12	8.9
	>1000	26	52	17	95	70.4

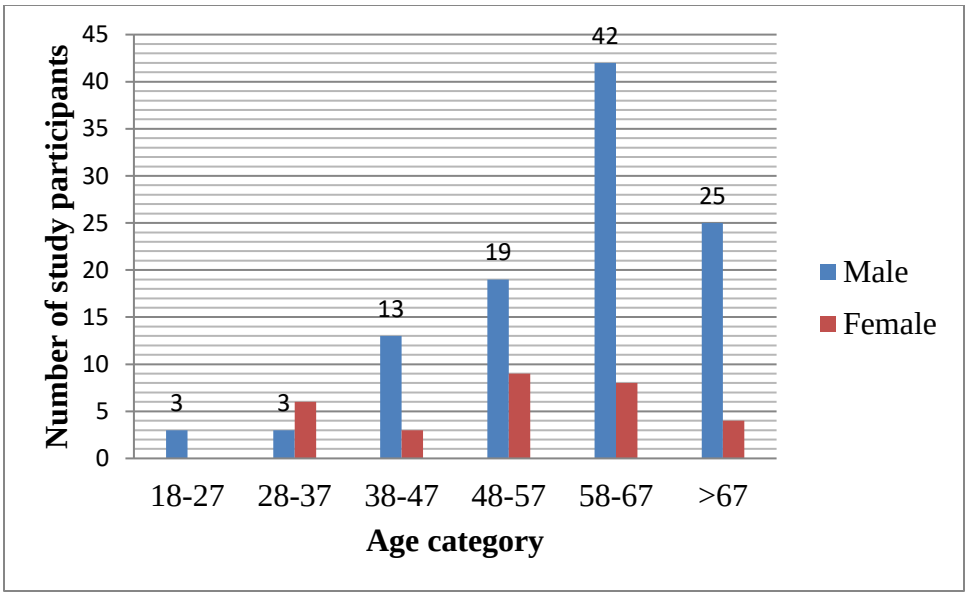

Figure 3. Age and sex distribution of patients investigated for Diabetic foot infection at selected public hospitals of Addis Ababa, Ethiopia. (May 2018 – April 2019)

7.2 Clinical, Behavioral and Educational Factors of DFI patients

Among the total 135 study participants, 120 (88.89%) had type 2 diabetes mellitus while the rest had type 1 DM. 68 (50.37%) of the patients were on oral hypoglycemic agents, 36 (26.67%) were treated with insulin and 31 (22.96%) were on both insulin and oral hypoglycemic agent. The BMI of most study participants was between 18.5 and 24.9 kg/m².The mean fasting blood glucose level among diabetic patients with foot ulcer was 255.62 mg/dl. Fifty five (40.74%) were diabetic for more than 10 years. Comorbidities with other diseases were common among participants, and among these, 80 (59.26%) participants were hypertensive, Forty-seven (34.81%) study participants had Retinopathy, Peripheral vascular disease was detected in 39 (28.89%) participants, 62 (45.93%) had peripheral neuropathy, Nephropathy was detected on 43(31.85%) . Similarly, 26 (19.26%) and 46(34.07%) of the study participants had dermatologic disease and foot deformity consecutively (Table 2).

Proper health education on diabetic foot self-care practice was given for only 42 (31.11%) of the participants. Fifteen (11.11%) were bare foot walkers. One hundred nine (80.74%) wear ill fitting shoes. Checking inside of the shoe before wearing were exercised by 68(50.37%) of the

participants. Sixty four (47.41%) inspected their feet for any changes, Sixty seven (49.63%) dried between toes after washing and 73(54.07%) had regular foot checkup. Thirty six (26.67%) of participants did not monitor their blood glucose level. Twenty three (17.04%) of the study participants were daily smokers. Forty (29.63%) study participants were alcoholic. Among those, 11 (8.15%) of study participants were highly alcoholic. Regarding involvement in physical exercise, 75(55.56%) of the participants were not engaged in physical activities.

Table 2. Clinical and behavioral data of Diabetic patients with infected ulcers (May, 2018 –April,2019)

Variables		DFI			Total Frequency	Percent
		Mild	Moderate	Severe		
Type of Dm	Type I	6	7	2	15	11.11
	Type II	30	68	22	120	88.89
Diabetic treatment modality	On insulin	8	20	8	36	26.67
	On Oral Hypoglycemic Agents	23	37	8	68	50.37
	Insulin & oral hypoglycemic drugs	5	18	8	31	22.96
BMI	18.5-24.9	25	41	12	78	57.78
	25-29.9	11	33	9	53	39.26
	> 30	0	1	3	4	2.96
Duration of Dm	<10 year	26	43	11	80	59.26
	>10 year	10	32	13	55	40.74
Duration of foot ulcer(month)	<1 month	16	24	2	42	31.11
	1-3 month	10	28	8	46	34.07
	>3 month	10	23	14	47	34.81
Duration on Antibiotic	No antibiotics	12	21	1	34	25.19
	Antibiotics with in past 30 days	24	54	23	101	74.81
Previous Ulcer	Yes	10	20	8	38	28.15
	No	26	55	16	97	71.85
previous Amputation	Yes	4	11	5	20	14.81
	No	32	64	19	115	85.19
Retinopathy	Yes	9	25	13	47	34.81
	No	27	50	11	88	65.19
Nephropathy	Yes	4	24	15	43	31.85
	No	32	51	9	92	68.15

	Yes	14	34	14	62	45.93
Peripheral Neuropathy	No	22	41	10	73	54.07
	Yes	5	17	17	39	28.89
Peripheral Vascular Disease	No	31	58	7	96	71.11
	Yes	15	46	19	80	59.26
Hypertension	No	21	29	5	55	40.74
	Dry	16	34	11	61	45.19
	Moist	16	33	11	60	44.44
Foot skin texture	Cracked	4	8	2	14	10.37
Deformity in shape or structure of of foot	Yes	13	26	7	46	34.07
	No	23	49	17	89	65.93
	Yes	7	15	4	26	19.26
Dermatologic Disease	No	29	60	20	109	80.74
	Yes	21	42	10	73	54.07
Regular foot check up	No	15	33	14	62	45.93
Education on proper foot care	Yes	19	20	3	42	31.11
	No	17	55	21	93	68.89
	Yes	27	63	19	109	80.74
Wearing ill fitting shoe	No	9	12	5	26	19.26
	Not monitor	11	14	11	36	26.67
	Daily	0	2	1	3	2.22
	Weekly	7	12	3	22	16.30
	Every 15 days	10	13	2	25	18.52
Frequency of checking Fbs	Monthly	8	34	7	49	36.30
Drying b/n toes after washing	Yes	21	37	9	67	49.63
	No	15	38	15	68	50.37
Checking inside of the shoe before wearing	Yes	23	35	10	68	50.37
	No	13	40	14	67	49.63
Inspection of feet for any change(Redness,Warmeth)	Yes	19	38	7	64	47.41
	No	17	37	17	71	52.59
	Yes	5	7	3	15	11.11
Bare foot walking	No	31	68	21	120	88.89
	Yes	12	35	13	60	44.44
Physical Activity	No	24	40	11	75	55.56
	Every Day	6	11	6	23	17.04
	Some day	0	1	0	1	0.74
Smoking frequency	Not at all	30	63	18	111	82.22
	No consumption	25	56	14	95	70.37
	Low (1-20 g/day)	5	11	4	20	14.81
Alcohol consumption per day for drunk	Moderate (21-40 g/day)	1	5	3	9	6.67
	High (≥ 41 g/day)	5	3	3	11	8.15

7.3. Logistic regression results

Table 3 shows the bivariate and multivariate regression analysis when Diabetic foot infection is regressed on different covariates. After controlling the effect of covariates, the regression analysis showed a statistically significant association between Diabetic foot infection and overweight/obesity. Specifically, we can conclude that individuals with normal BMI and overweight were associated with less sever Diabetic foot infections than Obese individual, [AOR 0.052; 95% CI: 0.004- 0.663 and AOR 0.072; 95% CI: 0.006- 0.935 respectively].

Moreover, PVD, Nephropathy, Health education on proper foot care were also found to be associated with Diabetic foot infection.

The odds of severe diabetic foot infection were less among diabetic patients without PVD than the odds With PVD, [AOR 0.298; 95% CI: 0.116- 0.765]. Similarly, the odds of having sever diabetic foot infection were less among adults without nephropathy than those having nephropathy, [AOR 0.354; 95% CI: 0.135-0.927].

Furthermore the odds of having sever diabetic infection among patients who had no health education on proper foot self care were significantly higher than the odds among those who had the health education, [AOR 3.743; 95% CI: 1.615-8.674].

Table 3. Results of the multiple POM using Diabetic Foot infection as response three ordered categories♣

Co-variable	Estimate	Standard error	P-value	COR(95%CI)	AOR(95% CI)
Classification = 0	-6.241	2.050	.002	NA	0.002(0.000-0.108)
Classification = 1	-2.643	1.954	.176	NA	0.071(0.002-3.279)
Antibiotics taken within the last one month [Those who took Antibiotics as Reference]					
No Antibiotics	.453	.431	.294	2.389(1.110-5.141)	1.573(0.675-3.668)
Duration of the Diabetes (years) [10+ years as reference]					
< 10 years	.448	.394	.256	2.063(1.045-4.072)	1.565(0.723-3.389)
Presence of Retinopathy [Absence as reference]					
Yes	-.029	.459	.950	0.450(0.222-0.912)	0.971(0.395-2.390)
Presence of Nephropathy [Absence as reference]					
Yes	-1.038	.491	**.034**	0.198(0.091-0.435)	**0.354**(**0.135-0.927**)
Presence of PVD [Absence as reference]					
Yes	-1.212	.482	**.012**	0.156(0.068-0.355)	**0.298**(**0.116-0.765**)
Presence of Hypertension [Absence as reference]					
Yes	-.500	.409	.222	0.357(0.178-0.715)	0.606(0.272-1.353)
Health Education on proper foot care [Not educated as reference]					
Educated	1.320	.429	**.002**	3.713(1.763-7.821)	**3.743**(**1.615-8.674**)
Participant's BMI [Obese (≥30) as reference]					
18.5-24.9	-2.950	1.295	**.023**	0.051(0.005-0.533)	**0.052**(**0.004-0.663**)
25-29.9	-2.624	1.305	**.044**	0.077(0.007-0.803)	**0.072**(**0.006-0.935**)
Duration of the foot ulcer(Month) [>3 months as reference]					
< 1	-.559	.478	.242	0.287(0.124-0.661)	0.572(0.224-1.459)
>3	-.122	.453	.787	0.667(0.299-1.484)	0.885(0.364-2.148)

Test of parallel line(proportional odds assumption): Chi-square = 9.835, df = 11, p-value =.545
Model fitting information: Chi-square = 56.233, df =11, p-value = 0.000,Goodness-of-Fit:Pearson(Chi-Square=160.147,df =183,p-value=0.887),Deviance(Chi-Square=158.008,df =183,p-value=.909)
= Pseudo R2 = .396

♣ Sample size:135

7.4. Bacterial profile of the Diabetic Foot Infections

Among the 135 patients recruited in this study, 85(62.96%) had mono-bacterial while 50 (37.04%) had mixed bacterial infections. Gram negative aerobic bacterial infections were more at 121(63.7%) than gram positive aerobic bacteria 69, (36.3%). The most commonly isolated microorganism was *S. aureus* (26.3%), followed by *Klebsiella spp* (22.1%), *Proteus spp* (11.1%), *E. coli* (10.5%) and Acinetobacter (10.5), Coagulase Negative *Staphylococcus* (CONS) (6.3%), Enterobacter clocae *(3.2%)*, *E. faecalis* (2.6%), *P. aeruginosa* (2.6%), *P.retgeri*(2.6%),*M. Morgani*(1.1%) and *Viridian streptococci*(1.1%). The proportion of each bacterial isolate to the total isolates is presented in Table 4.

Table 4. Magnitude of bacterial isolates from Diabetic foot infection at selected public hospitals from May,2018 to April, 2019.

Bacteria isolates	Frequency	Percent
Gram positive bacteria	**69**	**36.3**
Staphylococcus aureus	50	26.3
Coagulase negative *staphylococcus(CONS)*	12	6.3
Enterococcus spp	5	2.6
Viridian streptococci	2	1.1
Gram negative bacteria	**121**	**63.7**
Proteus spp.	21	11.1
Pseudomonas aeruginosa	5	2.6
Klebsiella spp.	42	22.1
Escherichia coli	20	10.5
Enterobacter clocae	6	3.2
Acinetobacter spp.	20	10.5
Providencia retigeri	5	2.6
Morganella morgani	2	1.1
Total	**190**	**100**

7.5. The Prescription pattern of Antimicrobials in the Study Population

Mostly combination of antimicrobials were used as a therapy. Ceftriaxone ,Metronidazole combined was the most frequently prescribed antimicrobials (32.6%) for DFIs followed by Cloxacilin (11.9%), Amoxicillin/clavulanate (8.9%), Vancomycin and ceftriaxone combined(8.1%), Ceftriaxon,Gentamycine,vancomycin &metronidazole combination(7.4%) Clindamycin and ceftriaxone(3.0%) , ciprofloxacin (2.2%), Vancomycin with Meropinem combined was the least prescribed(0.7%) . This is summarized in figure 4.

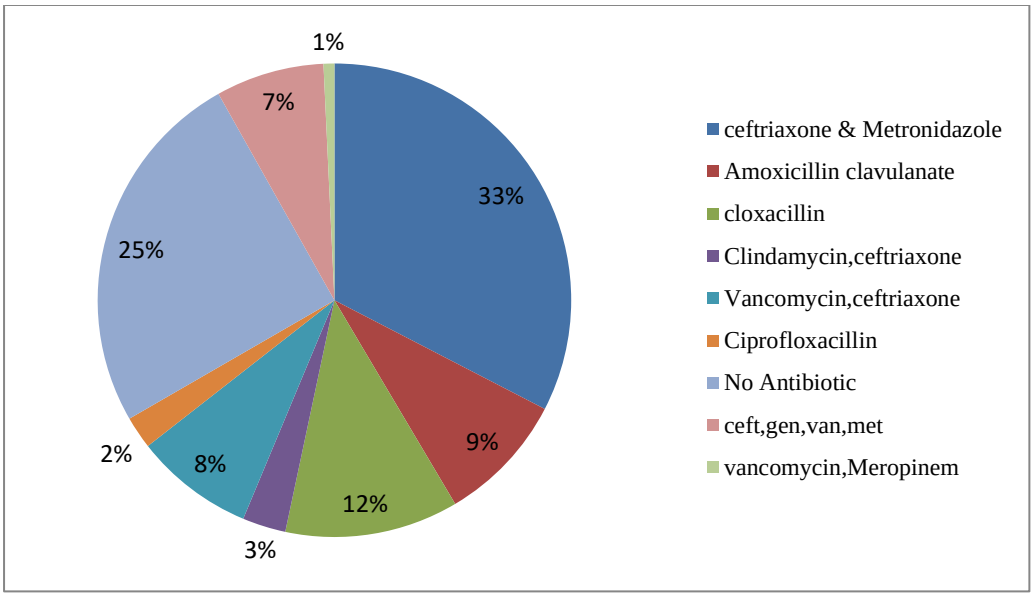

Figure 4 . The prescription pattern of Antimicrobials for DFI at selected public hospitals in Addis Ababa, Ethiopia from May, 2018 to April, 2019.

7.6 Antimicrobial susceptibility patterns of different bacterial isolates

The predominant S.*aureus* isolate among the gram positive isolates showed resistance for Penicillin (96%), Doxycycline (60%), Chloramphenicol (54%) and Erytromycin (50%) . Most of the CONS isolates showed resistance to peniciline (75%), Chloramphenicol (66.7%), Cotrimoxazole (66.7%) ,Doxycycline (66.7%). 58.3% resistance was seen to Cefoxitin, clindamycin, Erytromycin, ciprofloxacin and Torbamycin. *Enterococcus spp* exhibited resistance against ampicillin (40%) and vancomycin (20%). On the other hand, *Viridian Streptococci* 50% Sensitivivity level was seen on penicillin ,erythromycin ,clindamycin and ampicillin (Table 6).

Among Gram negative isolates, All of the isolates showed the highest sensitivity against Amikacin (85%-100 %) and Meropenem (72.2%-100%) except for *Acinetobacter* which showed only 65% & 40% sensitivity for both antibiotics consecutively. All gram negative isolates showed high resistance for Ampiciline (100%). *Morganella Morgani* was highly resistant (100%) for Augmentine. Resistance to second and third generation cephalosporins (cefotaxime,cefuroxime,ceftriaxone and ceftazidime) was observed for *Klebsiella spp* (87.5%-100%), *Enterobacter Spp.* (83.3%). *Acinetobacter Spp* showed high level of resistance for most antibiotics like ceftazidime and Tazobactam-peperazine(100%),90% for cotrimoxazole and cefepime (Table 7).

Table 5. Antimicrobial susceptibility pattern of Gram positive bacterial isolates from DFIs at Selected public hospitals in Addis Ababa, Ethiopia from May, 2018 to April, 2019.

Isolates (n)		Antimicrobial agents in number (%)											
		FOX	P	E	CN	VAN	SXT	AMP	GEN	CHL	CIP	DOX	TORB
S.Aureus	S	31(62)	2(4)	23(46)	30(60)	NA	27(54)	NA	25(50)	26(46)	28(50)	20(40)	25(50)
(n=50)	I	0(0)	0(0)	2(4)	1(2)	NA	1(2)	NA	1(2)	0(0)	3(4)	0(0)	2(4)
	R	19(38)	48(96)	25(50)	19(38)	NA	22(44)	NA	24(48)	24(54)	19(46)	30(60)	23(46)
CoNS	S	5(41.7)	3(25)	5(41.7)	5(41.7)	NA	4(33.3)	NA	5(41.7)	4(33.3)	5(41.7)	4(33.3)	5(41.7)
(n=12)	I	0(0)	0(0)	0(0)	0(0)	NA	0(0)	NA	0(0)	0(0)	0(0)	0(0)	0(0)
	R	7(58.3)	9(75)	7(58.3)	7(58.3)	NA	8(66.7)	NA	7(58.3)	8(66.7)	7(58.3)	8(66.7)	7(58.3)
Enterococcus	S	NA	NA	NA	NA	0(0)	NA	3(60)	NA	NA	NA	NA	NA
spp.	I	NA	NA	NA	NA	4(80)	NA	0(0)	NA	NA	NA	NA	NA
spp.(n=5)	R	NA	NA	NA	NA	1(20)	NA	2(40)	NA	NA	NA	NA	NA
Viridian	S	NA	1(50)	1(50)	1(50)	NA	NA	1(50)	NA	NA	NA	NA	NA
streptococci	I	NA	0(0)	0(0)	0(0)	NA	NA	0(0)	NA	NA	NA	NA	NA
spp.(n=2)	R	NA	1(50)	1(50)	1(50)	NA	NA	1(50)	NA	NA	NA	NA	NA

Key:- FOX=Cefoxitin, P=Penicillin, E=Erythromycin, CN=Clindamycin, CIP=Ciprofloxacillin, GEN=Gentamycin, VAN=Vancomycin, CHL=Chloramphenicol, SXT=Cotrimoxazole, P=Penicillin, AUG=Augumentin, S=Sensitive, I=Intermediate, R=Resistance,n=number AST= Antimicrobial susceptibility testing.

Table 6. Antimicrobial susceptibility pattern of Gram negative bacterial isolates from DFIs at Selected public hospitals in Addis Ababa, Ethiopia from May, 2018 to April 2019.

Isolates	P	Antimicrobial Agents in No. (%)														
		SXT	AMP	AMK	GEN	CHL	CIP	CRO	CTX	CAZ	AUG	CFP	MEM	TZP	CFX	TORB
Proteus mirabilis	S	2 (28.6)	0	6 (85.7)	5 (71.4)	5 (71.4)	5 (71.4)	4 (57.1)	4 (57.1)	4 (57.1)	2 (28.6)	5 (71.4)	7 (100)	5 (71.4)	4 (57.1)	5 (71.4)
	I	0	0	0	0	1 (14.3)	0	0	0	0	0	1 (14.3)	0	0	0	0
	R	5 (71.4)	7 (100)	1 (14.3)	2 (28.6)	1 (14.3)	2 (28.6)	3 (42.9)	3 (42.9)	3 (42.9)	5 (71.4)	1 (14.3)	0	2 (28.6)	3 (42.9)	2 (28.6)
Proteus vulgaris	S	9 (60)	0	15 (100)	13 (86.7)	7 (46.7)	13 (86.7)	10 (66.7)	10 (66.7)	10 (66.7)	6 (40)	12 (80)	15 (100)	12 (80)	10 (66.7)	13 (86.7)
	I	0	0	0	0	1 (6.6)	0	1 (6.6)	0	0	1 (6.7)	0	0	1 (6.7)	0	0
	R	6(40)	15 ((100)	0	2 (13.3)	7 (46.7)	2 (13.3)	4 (26.7)	5 (33.3)	5 (33.3)	8 (53.3)	3 (20)	0	2 (13.3)	5 (33.3)	2 (13.3)
klebsiella pneumoniae	S	8 (44.4)	0	18 (100)	15 (83.3)	11 (61.1)	14 (77.8)	0	0	0	0	10 (55.6)	13 (72.2)	3 (16.7)	0	16 (88.9)
	I	0	0	0	0	0	0	0	0	0	0	0	0	2 (11.1)	0	0
	R	10 (55.6)	18 (100)	0	3 (16.7)	7 (38.9)	4 (22.2)	18 (100)	18 (100)	18 (100)	18 (100)	8 (44.4)	5 (27.8)	13 (72.2)	18 (100)	2 (11.1)
klebsiella Oxytoca	S	4 (26.7)	0	13 (86.7)	8 (53.3)	9 (60)	8 (53.3)	2 (13.3)	2 (13.3)	2 (13.3)	2 (13.3)	7 (46.7)	14 (93.3)	5 (33.3)	4 (26.7)	8 (53.3)
	I	0	0	0	0	0	0	0	0	0	0	2(13.3)	0	3(20)	0	0
	R	11 (73.3)	15 (100)	2 (13.3)	7 (46.7)	6 (40)	7 (46.7)	13 (86.7)	13 (86.7)	13 (86.7)	13 (86.7)	6 (40)	1 (6.7)	7 (46.7)	11 (73.3)	7 (46.7)
Klebsiella ozenae	S	2 (25)	0	7 (87.5)	6 (75)	6 (75)	5 (62.5)	1 (12.5)	1 (12.5)	1 (12.5)	0	5 (62.5)	7 (87.5)	2 (25)	1 (12.5)	7 (87.5)
	I	0	0	0	0	0	0	0	0	0	0	1 (12.5)	0	2 (25)	0	0
	R	6 (75)	8 (100)	1 (12.5)	2 (25)	2 (25)	3 (37.5)	7 (87.5)	7 (87.5)	7 (87.5)	8 (100)	2 (25)	1 (12.5)	4 (50)	7 (87.5)	1 (12.5)
Escherichia	S	4	0	19	16	11	10	7	7	7	5	9	20	11	7	18

coli		(20)		(95)	(80)	(55)	(50)	(35)	(35)	(35)	(25)	(45)	(100)	(55)	(35)	(90)
	I	0	0	1 (5)	0	1 (5)	1 (5)	0	1 (5)	1 (5)	0	1 (5)	0	0	1 (5)	0
	R	16 (80)	20 (100)	0	4 (20)	8 (40)	9 (45)	13 (65)	12 (60)	12 (60)	15(75)	10 (50)	0	9(45)	12 (60)	2 (10)
Pseudomonas aeruginosa	S	NA	NA	5 (100)	5 (100)	NA	3 (60)	NA	NA	2 (40)	NA	4 (80)	5 (100)	3 (60)	NA	5 (100)
	I	NA	NA	0	0	NA	0	NA	NA	1 (20)	NA	1 (20)	0	1 (20)	NA	0
	R	NA	NA	0	0	NA	2 (40)	NA	NA	2 (40)	NA	0	0	1 (20)	NA	0
Acinetobacter spp.	S	2 (10)	NA	13 (65)	11 (55)	NA	4 (20)	NA	NA	0	NA	1(5)	8 (40)	0	NA	10 (50)
	I	0	NA	1(5)	0	NA	1(5)	NA	NA	0	NA	1(5)	0	0	NA	0
	R	18 (90)	NA	6 (30)	9 (45)	NA	15 (75)	NA	NA	20 (100)	NA	18 (90)	12 (60)	20 (100)	NA	10 (50)
Providencia rettgeri	S	2 (40)	0	5 (100)	3 (60)	3 (60)	3 (60)	3 (60)	4 (80)	4 (80)	2 (40)	5 (100)	5 (100)	4 (80)	4 (80)	4 (80)
	I	0	0	0	0	0	0	0	0	0	0	0	0	1(20)	0	0
	R	3 (60)	5 (100)	0	2 (40)	2 (40)	2 (40)	2 (40)	1 (20)	1 (20)	3 (60)	0	0	0	1 (20)	1 (20)
Morganella morgani	S	1 (50)	0	2 (100)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	1 (50)	0	2 (100)	2 (100)	1 (50)	1 (50)	1 (50)
	I	0	0	0	0	0	1(50)	0	0	0	0	0	0	0	0	0
	R	1 (50)	2 (100)	0	1 (50)	1 (50)	0	1 (50)	1 (50)	1 (50)	2 (100)	0	0	1 (50)	1 (50)	1 (50)
Enterobacter cloacae	S	6 (100)	0	6 (100)	6 (100)	6 (100)	5 (83.3)	1 (16.7)	1 (16.7)	1 (16.7)	1 (16.7)	5 (83.3)	6 (100)	1 (16.7)	1 (16.7)	6 (100)
	I	0	0	0	0	0	0	0	0	0	0	0	0	3(50)	0	0
	R	0	6 (100)	0	0	0	1 (16.7)	5 (83.3)	5 (83.3)	5 (83.3)	5 (83.3)	1 (16.7)	0	2 (33.3)	5 (83.3)	0

AMP: Ampicillin; CRO: Ceftriaxone; AUG: Amoxicillin+ clavulanic acid; CHL: chloroamphenicol; GEN: gentamicin; CAZ: Ceftazidime; CTX: Cefotaxime; SXT: trimethoprim-sulphamethoxazole; MEM: Meropenem; CIP: ciprofloxacin; AMK;Amikacin CFP: cefepim; TZP: Piperacillin-tazobactam; TORB: Tobramycin; CFX:Cefuroxime;NA: Not Done; P: Pattern *S: Sensitive; I: Intermediate; R:Resistant

7.7. Multi-drug resistant isolates

Antibiogram of the isolates in this study showed that 100% multidrug resistance among *Klebsiella pneumoniae* , *Klebsiella ozenae* ,*Morganella morgani and Acinitobacter* spp . Gram positive isolates S. *aureus* 30(60 %) ,*CoNS* 8(67%) and Viridian streptococci 1(50%) of isolates were resistant to four and more antibiotics. On the other hand, among gram negative isolates *Pseudomonas aeruginosa* 1 (20%), *E. coli species*17(85%),*P. mirabilis* 5(71.43%),*P. vulgaris* 8(53.33%),*P.retigeri* 3(60%) showed resistance to three antibiotics. Majority of isolates in *Klebsiella oxytoca* 13(86.6%) and *Entrobacter cloacae* 5(83.33%) were resistant to six up to ten antibiotics. In general, 140(73.68%) of the isolates in our study developed multidrug resistance to at least one drug in three different classes of antibiotics (≥3 antibiotics)

Table 7. Antibiogram of bacteria isolated from patients with Diabetic foot infections at selected public hospitals from May, 2018 to April, 2019.

Bacterial isolates	**No. (%) of resistance**							
	R0	**R1**	**R2**	**R3**	**R4**	**R5**	**R6-10**	MDR
Staphylococcus aureus (n=50)	2(4)	16(32)	2(4)	0	2(4)	3(6)	25(50)	30(60)
CoNS(n=12)	2(17)	2(17)	0	0	1(8)	0	7(58)	8(67)
Enterococcus spp.(n=5)	3(60)	1(20)	1(20)	0	0	0	0	1(20)
Viridian streptococci(n=2)	1(50)	0	0	0	1(50)	0	0	1(50)
klebsiella pneumoniae (n=18)	0	0	0	0	0	0	18(100)	18(100)
klebsiella Oxytoca(n=15)	0	1(6.7)	1(6.7)	0	0	0	13(86.6)	13(86.6)
Klebsiella ozenae(n=8)	0	0	0	0	1(12.5)	0	7(87.5)	8(100)
Escherichia coli(n=20)	0	2(10)	1(5)	2(10)	2(10)	0	13(65)	17(85)
Proteus vulgaris(n=15)	0	3(20)	4(26.67)	3(20)	0	0	5(33.33)	8(53.33)
Proteus mirabilis(n=7)	0	1(14.29)	1(14.29)	2(28.57	0	0	3(42.85)	5(71.43)
Pseudomonas aeruginosa (n=5)	2(40)	2(40)	0	1(20)	0	0	0	1(20)
Entrobacter Cloacae(n=6)	0	1(16.67)	0	0	0	0	5(83.33)	5(83.33)
Providencia retigeri(n=5)	0	1(20)	1(20)	1(20)	0	0	2(40)	3(60)
Morganella Morgani(n=2)	0	0	0	1(50)	0	0	1(50)	2(100)
Acinetobacter spp.(n=20)	0	0	0	0	0	0	20(100)	20(100)
Total	**10(5.3)**	**30(15.7)**	**11(5.8)**	**10(5.3)**	**7(3.7)**	**3(1.6)**	**119(62.6)**	**140(73.7)**

Key ; R0 = no resistance to antibiotic ,R1= resistance to 1 antibiotics, R2= resistance to 2 antibiotics ,R3=resistance to 3 antibiotics, R4 =resistance to 4 antibiotics, R5 =resistance to 5 antibiotics , R 6-10=resistance to 6-10 antibiotics

7.7.1 Methicillin resistant Staphylococcus aureus (MRSA)

All isolates resistant to cefoxitin were classified as MRSA. In our study susceptibility test of cefoxitin disk out of 50 *Staphylococcus aureus* isolates revealed that 19(38%) were resistant to cefoxitin (MRSA).

7.7.2 Carbapenem resistance

Out of 121 Gram negative isolates 96 (79.34%) were *Enterobacteriaceae.*7(7.3%) of *Enterobacteriaceae(CRE)* were Carbapenem resistant producing KPC and All of them were from *Klebsiella* species. Carbapenem resistance were also identified in other gram negative species like *Acinetobactr* species 12 (9.9%).

7.7.3 Extended spectrum beta-lactamase (ESBL)

Combined disk(double disk potentiate) test and Double disk synergy(DDST) methods were implemented for phenotypic confirmation of ESBL and concordant result was found for all isolates. Among 96(79.34%) *Enterobacteriaceae* isolates 12(12.5%) were found to be ESBL producer. *Klebsiella* species *6(50%)* being the predominant ESBL producer (*Klebsiella pneumoniae* 3 (25%) , *Klebsiella ozenae 2(16.67%)* , *Klebsiella oxytoca 1(8.33%))* followed by *Escherichia coli* 4 (33.3%) and *proteus* species 2(16.7%). *proteus mirabilis* and *proteus vulgaris* were among the *proteus* species being ESBL producers.

7.7.4 Vancomycin resistant Entrococci (VRE)

All *Enterococcus* isolates were examined for reduced vancomycin susceptibility by EUCAST Disk diffusion method. Our result showed 1(50%) resistance and 1 (50%) sensitivity.

8. Discussion

DFIs are a common and serious complication of diabetes which is present in up to 50% of DFUs, and 80% of non-traumatic lower-limb amputations are a consequence of DFU infection [52,53]. The bacterial etiologies and risk factors associated with the Diabetic foot infection are not well studied and published information is scarce in Ethiopia. Therefore, the present study was undertaken to identify and characterize bacterial etiologies of Diabetic foot infection, to determine their susceptibility pattern and the different factors associated with severity of infections.

Among 135 clinical specimens collected from patients with diabetic foot infection, bacteria were identified in all patients. In this study, a total of 190 microorganisms were isolated from 135 patients, with an average of 1.41 microorganisms isolated from each patient.This is quite different to hospital based study conducted in Ethiopia by Amogne w et al, [25] where cultures yielded an average of 0.77 organisms per case. However, this study has similar proportion with studies conducted in Malaysia, Saudi Arabia, and southern Iran in the rate of 1.47, 1.45 and 1.42, respectively [54,38,55]. Studies from abroad showed similar bacterial proportion may be due to bacteria isolates were from infected ulcers however the local study was conducted generally on DFUs.

In the present study, 62.96 percent of cases had monomicrobial infection, similar to other studies conducted in Turkey, Egypt and Ethiopia [34, 40, 28&25], but different from study in USA, Cameroon and Nigeria [13, 39 & 56]. Similarities in socio demographic characteristics of study participants among the studies may contribute for consistent prevalence with studies conducted within Ethiopia. The discrepancy here with the other studies may be due to that of anaerobes were not included in our study and monobacterial infections were found to be more prevalent.

Spectrums of bacteria vary widely in diabetic foot infections. In our study, Gram negative bacteria were isolated predominantly 121 (63.7%), while Gram positive cocci accounted for 69(36.3%). The most commonly isolated microorganism was *S. aureus* (26.3%), followed by *Klebsiella spp* (22.1%), *Proteus spp* (11.1%), *E. coli* (10.5%) and Acinetobacter (10.5), Coagulase Negative *Staphylococcus* (CONS) (6.3%), Enterobacter clocae *(3.2), E. faecalis*

(2.6%), *P. aeruginosa* (2.6%), *P.retgeri*(2.6),*M. Morgani*(1.1) and *Viridian streptococci*(1.1). This is in agreement with the previous study done on diabetic infections at TASH by Feleke Y. et al,[28]. Similar finding has been published elsewhere [36,41,42,57,58,59]. However it is somehow different from the retrospective study conducted on diabetic foot in TASH by Amogne W. et al.,that indicated *Klebsiella species* to be the predominant bacteria [25]. This contradiction reinforces the fact of variability of organisms infecting DFUs across different regions and even within the same settings and at different times as has been demonstrated in studies [40].The variation with the local study may also be related to difference in the method and the time gap between the studies which can result in the change in the spectrum and the Antibiogram of the isolates.

In this study, Gram positive and Gram negative bacteria showed decreased sensitivity to most of the antimicrobial agents tested (Tables 5 and 6). The predominant gram positive bacteria isolate was *S. aureus.* This was consistent with the reviewed articles on microbiology and antimicrobial therapy by Kwon KT *et al.* [60] in Korea. *Staphylococcus aureus* showed the highest sensitivity to cefoxitine (62%) followed by clindamycin (60%). In our study S.*aureus* showed resistance for Penicillin (96%), Doxycycline (60%), Chloramphenicol (54%) and Erytromycin (50%). This is an indication of the alarming levels of resistance for different group of Antibiotics by *S. aureus* at the hospitals. This is contrary to the findings in Egypt by Hefni et al [40] where S. aureus showed high sensitivity to Chloramphenicol, Erythromycine and Tetracycline but similar to the findings by Hena et al. [61] and Rama Ramani et al.[62] in India who reported a resistance to penicillin.

In Diabetic patients the impaired host defenses around necrotic soft tissue or bone may allow Low virulence colonizing bacteria, such as coagulase negative *Staphylococci* and *Corynebacterium* species (diphtheroids), to have pathogenic effects [63].Our study also indicated Coagulase Negative *Staphylococcus* (CoNS) (6.3%) as the second most prevalent bacteria followed by *E. faecalis* (2.6%) and *Viridian streptococci*(1.1). This result is close to the study conducted in Egypt where Coagulase-Negative *Staphylococci* (CoNS) 9.7% was the succeder to the most prevalent gram positive cocci *Staphylococcus aureus* [41]. Most of the CoNS isolates showed resistance to peniciline (75%), Chloramphenicol (66.7%), Cotrimoxazole (66.7%) ,Doxycycline (66.7%). 58.3% resistance was seen to Cefoxitin, clindamycin,

Erytromycin, ciprofloxacin and Tobramycin. This finding is not comparable with other study from india [64]. This emanated possibly from the differences in the sociodemographic, severity level or grades, health care sytem and methods used during sample collection. The antimicrobial susceptibility testing, also showed that vancomycin(80%) was the most effective against *Enterococcus faecalis*. This is inline with previous study done in Iran where most of the isolate were sensitive to Vancomycin [34].

Gram negative Aerobes were the leading in our study comprised 63.7% (121 of 190) of the total isolates. A Study from Egypt and Turkey also showed similar reports in which Gram-negative bacteria were more isolated at 67% and 61.3% compared with Gram-positive bacteria [40, 34].The concordance between the studies could be due to similarities in type of sample and the methods implemented. Our findings showed members of the *Enterobacteriaceae* family were the predominant group among the gram negative Aerobes in line with other study from brazil [32].The second ,third and fourth major isolates were *Klebsiella spp* (22.1%), *Proteus spp* (11.1%) and *E. coli* (10.5%) species. Considerable shares were also possessed by other isolate from the family like, *Enterobacter clocae (3.2%),P.retgeri*(2.6%) and *M. Morgani*(1.1%).

With regard to the susceptibility patterns, Meropinem and amikacin appeared to be the best antibiotics for therapy against Gram-negative organisms. All gram negative isolates showed high resistance for Ampiciline (100%). Morganella Morgani was highly resistant (100%) for Augmentine. Resistance to second and third-generation cephalosporins (cefotaxime, cefuroxime, ceftriaxone and ceftazidime) was observed for *Klebsiella spp* (87.5%- 100%) and *Enterobacter Spp.* (83.3%). Studies in Kuwait, Cameroon and Egypt also supported the finding most effective treatments for the Gram negative bacteria were Amikacin and imepinem [37, 39, 40].

Apart from the 75.9% *Enterobacteriaceae* family, other gram negative rods harbored significant part of diabetic foot infections. *Acinetobacter species* accounts for about 20(10.5%) of the total isolates in our study.Similar results has also been reported in Egypt (10.2%) and Iran(11%) [40, 65]. *Acinetobacter species* showed high level of resistance for most antibiotics like ceftazidime and Tazobactam-peperazine(100%) and 90% for cotrimoxazole and cefepime. Amikacin was found to be the better treatment option having 65% sensitivity. Our result generated similar

report with study conducted in India, which showed high level of resistance and the choice of antibiotic with better sensitivity was Amikacin (83%) [66].

The other aerobic gram negative bacilli less frequently isolated in our study was *Pseudomonas aeruginosa*, accounted for 2(2.6%). This outcome is contrary to that of Feleke et al, where 15% prevalence was registered .A possible explanation for this might be that of differences in the source of infection, use of Antibiotic for treatment , time gap between the studies and may also be due to the previous study was conducted solely on inpatient set up. The prevalence observed in this investigation are also far below those observed by Turhan et al .,in Turkey (29.8%)[34] and Parsa and Samani et al., in Iran (35%) [67].This discrepancies could be attributed to diverse source of infection, use of antibiotic treatment, sampling methods, regional differences, and the type and severity of infection. Our study showed great sensitivity to Antibiotics of the Aminoglycoside group (Amikacin, Gentamycin, Tobramycin) and Meropenem similar to other studies [40, 68]. Although *Pseudomonas* was known as a common invasive pathogen in DFIs causing severe tissue damage and exhibiting a high resistance to broad spectrum antibiotics [69],In our study it exhibited resistance only towards ceftazidime.

Antibiotic resistance has been observed all over the world for a broad range of microorganisms with an increasing prevalence that threatens human health [70]. In general, 140(73.68%) of the isolates in our study developed multidrug resistance to at least one drug in three different classes of antibiotics. This finding was close to MDR rate reported by Vimalin and Growther [61] in india and also with a study from Pakistan by Hayat et al. [71]. All reported more than two third of the isolated organisms were MDR. Multi drug resistant organisms are associated with more complications and longer hospital stay [41]. The most important MDROs isolated in our study were MRSA, ESBL producing gram negative rods, carbapenamase producing gram negative bacilli and vancomycin resistant Entercoccus.

The antibiotic resistance of staphylococci makes the treatment of different infections a therapeutic problem [72]. Susceptibility test of cefoxitin disk out of 50 *Staphylococcus aureus* isolates in our study revealed that 19(38%) were resistant to cefoxitin (MRSA). This result is in agreement with reports in Iran (38.5%) [73], North India (43.2%) [74] , UK(30.2.%) [75] and Saudi Arabia (30%) [38].This report was higher than that of the 4.4% prevalence from United States [13] but lower than a 56% reported from India [58]. This elaborates the fact that various

rate of MRSA detection in different research projects. It could be due to differences in socio demographics, widespread use of broad spectrum antibiotics, Severity of infection, Adherence to infection control measures, previous hospitalization, and isolation technique and there might be specific risk factor which was not covered in our study that might influence the isolation rate.

Carbapenamase producing gram negative bacilli were one of the emerging challenge when considering successful Antibiotic therapy. Significant number of isolates were found producing Carbapenamase. 7(7.3%) of *Enterobacteriaceae(CRE)* were Carbapenem resistant and all of them were from *Klebsiella* species. Carbapenem resistance was also identified in other gram negative species like *Acinitobactor species* 12 (9.9%). Over all carbapenamase producing gram negative rods in this study accounted 15.7%. This was inconsistent with the two studies in India in which carbapenemase producers were 31% & 16.5% of *enterobacteriaceae,* respectively [76,77].

The other MDROs isolated in our study were ESBL producing gram negative rods. Extended-spectrum β-lactamases (ESBLs) are a rapidly evolving group of enzymes which have the capability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid. Among 96(79.34%) *enterobacteriaceae* isolates 12(12.5%) were found to be ESBL producer. *Klebsiella species 6(50%)* being the predominant ESBL producer (*Klebsiella pneumoniae* 3 (25%) , *Klebsiella ozenae 2 (16.67%) , Klebsiella oxytoca 1 (8.33%))* followed by *Escherichia coli* 4 (33.3%) and *proteus species* 2 (16.7%). *proteus mirabilis* and *proteus vulgaris* were among the *proteus species* being ESBL producers. Culture result by Katz et al., [78] revealed that ESBL enzymes were produced by 11% of *Enterobacteriaceae* isolates consistent with our result and relatively lower when compared to study by Saltoglu et al., in Turkey [79] that isolated about 20%. We also noted a concurrent result regarding proportion of the *Klebsiella* spp. and E. coli isolates that were positive for ESBLs with studies from India .[80,81]

The last MDRO isolated in this study was VRE. Our result showed resistance to Vancomycin among *Enterococcus* species. Similarly a study from Iran indicated resistance to vancomycin among *Enterococcus* species isolates is emerging. Prevalence of 20.6% was indicated on their report. Few number of *Enterococcus* species isolates in our study population limited our ability to evaluate factors. However isolation of this organism by itself is essential in transferring

message efforts to preventing VRE's spread are paramount, indicating the microbial profile and VRE covering Antibiotics during treatment need to be taken in to account in our study sites in particular.

Among the 135 patients that were involved in our study, we have also evaluated the potential risk factors for Diabetic foot infections. By implementing Proportional odds model multivariate analysis, we found four statistically significant independent risk factors for Diabetic foot infection: PVD, Nephropathy, Health education on proper foot self care and BMI.

Foot infections in diabetic patients are an increasingly common problem and can have serious consequences. Different factors like the continued rise of incidence of diabetes in developed countries, especially in many underdeveloped countries, the weight gain and increased life expectancy of many diabetics have contributed to the increase in this problem [6].This strongly magnifies our finding that after controlling the effect of covariates, the regression analysis showed a statistically significant association between Diabetic foot infection and overweight/obesity. Specifically, we can conclude that individuals with normal BMI and overweight were associated with less sever Diabetic foot infections than Obese individual, [AOR 0.052; 95% CI: 0.004- 0.663 and AOR 0.072; 95% CI: 0.006- 0.935 respectively].

The current study found a Negative association between Diabetic foot infection and PVD. The odds of getting severe diabetic foot infection were less among diabetic patients without PVD than the odds With PVD, [AOR 0.298; 95% CI: 0.116- 0.765]. These result corroborates the findings of a great deal of the previous work in a large prospective study by Lavery et al., [44] who stated that the presence of PVD as one of the significant independent risk factors which increased two fold risk of foot infection. Another retrospective review also confirms that risk factors for Diabetic foot infection were previous amputation, peripheral vascular disease and neuropathy [45]. It is also encouraging to compare this association with that found by Chang BB et al. who stated that foot ischemia certainly appears to be associated with an increased severity of an infection [82].

With regard to Nephropathy, the odds of having sever diabetic foot infection were less among adults without nephropathy than those having nephropathy, [AOR 0.354; 95% CI: 0.135-0.927]. our data was similar to that reported by Margolis DJ et al., in U.K[83] an observational study

reflected that, there is a strong association between stage of CKD and DFU or Lower Extrimity Amputation (LEA) that is probably not just related to the presence of peripheral arterial disease. Individuals with even moderate CKD (eGFR <60 ml/min per 1.73 m2) have an increased risk for DFU and LEA.This is also further supported by other studies , they have shown the risk of LEA is at least two to six times greater among those with both diabetes and CKD than among those with diabetes alone [84,85].

It is most imperative for Diabetic patients to be educated and made aware regarding the complications of diabetic foot and knowledge to take care of foot. Lack of education on proper foot self care has been the major problem identified in our study. The odds of having sever diabetic infection among patients who had no health education on proper foot self care were significantly higher than the odds among those who had the health education, [AOR 3.743; 95% CI: 1.615-8.674].This study supports evidence from other studies elsewhere [86,87,88].The studies concluded that patients who reported having foot infections and wound were the ones who lack awareness regarding diabetic foot complications and foot care and really did not follow good foot care practices.

9. Strength and limitation of the study

9.1 strength of the study

- The emerging MDROs like MRSA, VRE, ESBL producing GNR, Carbapenemase producing Gram negative bacilli were isolated.
- Recommendation on the most effective Antimicrobials were done
- Multiple Ordinal regressions Analysis on DFI for identification of risk factors were performed which was not previously addressed in Ethiopia and other countries.

9.2 Limitations of the study

- No anerobic culture was performed
- Ability to generalize our findings may be affected in a hospital based study
- Since Convenient sampling method was used equal chance to all Diabetic patients might not be given
- It is difficult to draw cause effect relationships in cross sectional studies.

10. Conclusion

Lack of awareness creation on the complications of diabetics and proper self foot care has been the major contributing factor to severity of foot infections in the diabetics in addition to PVD, BMI and nephropathy.

A total of 190 bacterial isolates were identified in this study with gram negative bacteria being the predominant. Diabetic foot infections were associated with monomicrobial etiology.

In general, 73.68% of the isolates in our study developed multidrug resistance to at least one drug in three different classes of antibiotics. Most of the antimicrobial used empirically in this study were not effective for the DFIs. The antimicrobial susceptibility patterns for each bacterium differ at different time intervals and hence appropriate management of diabetic foot infections would necessitate identification of the causative bacteria and performing susceptibility patterns at that point in time so as to reduce antimicrobial resistance.

With regard to the susceptibility patterns, Meropinem and Amikacin appeared to be the best antibiotics for therapy against gram negative organisms.The antimicrobial susceptibility testing also showed that Vancomycin and Cefoxitin were still the effective Antimicrobials against the Gram positive organisms.

11. Recommendations

Optimally, definitive therapy should be based upon culture and susceptibility analysis to promote the rational use of the agents and reduce emergence of bacterial resistance to antimicrobials.

For Mild Diabetic foot infections it would be better to delay initiation of antibiotics until culture and sensitivity test results are obtained.

Empirical treatment for moderate to severely infected diabetic foot ulcers at public hospitals in Addis Ababa may be considered with Amikacin, meropenem, Vancomycin and cefoxitin prior to culture and sensitivity test results.

Antimicrobial surveillance and susceptibility patterns of the isolates from DFIs regularly so as to guide the empiric use of antimicrobial agents.

Large scale research should be carried to determine the sensitivity patterns of anaerobic organisms infecting DFUs.

Establishing a primary prevention programme and implementing a multidisciplinary approach to detect the at risk foot early and avoid late presentation and complicated ulcers is recommended.

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13.Annexes

Annex 1: Information sheet

Study title: Bacterial profile and Antimicrobial susceptibility pattern of common bacteria isolates from diabetic foot infections at selected public Hospitals of Addis Ababa, Ethiopia.

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Ethical approval: TASH/ ALERT /St paul's Hospital/Addis Ababa Health Bureau/ University of Addis Ababa Ethics and Research Committee.Addis Ababa. Tel; +251 11 275 5170. AAERC/ AHRI's address; tel +(251)113483752 fax +(251)113211563

Introduction: My name is Bereketeab Berhanu and I am a postgraduate student in the Department of Medical Laboratory Sciences, College of health sciences, Addis Ababa University, Addis Ababa, Ethiopia . I am pursuing a degree of Master in Clinical Laboratory Sciences (Diagnostic and Public Health Microbiology). I am conducting a study on Bacterial profile and Antimicrobial susceptibility pattern of common bacteria isolates from diabetic foot infections at selected public hospitals of Addis Ababa, Ethiopia.

Purpose of the study: This study aims to assess the Bacterial profile and antimicrobial agents used to treat infected diabetic foot ulcers and their sensitivity patterns among adult patients at TASH, ALERT, St. Paul's,Yekatit 12,Zewditu,Ras Desta and Minilik Memorial Hospitals. The results of this study will aid in the choice of the most suitable antimicrobial for the infected diabetic foot ulcer.

Your role: Your role in this study should you agree to participate shall be to answer some questions which will be asked by the principal investigator guided by a questionnaire. You will also have a wound swab obtained from your diabetic foot ulcer for a laboratory test. Your inclusion in this study is purely at your own will and you have a right to decline to participate without any consequences or penalty. If you agree to participate in the study, you are still free to pull out at any one point for whatever reason without any consequences or penalties.

Risk and discomfort: There is no risk involved in taking the wound swab or the Aspirate except for a slight touch of the cotton swab that will be felt at the point of swabbing or the needle at the puncture site. Good clinical and laboratory guidelines will be practiced during the collecting and handling of the wound swab/Aspirate from you to minimize any risks.

Benefits: You may not benefit from this study immediately but the results of this study will aid in the choice of the most effective antimicrobial agent to use in case there is infection in the foot ulcer.

Cost: You will not incur any costs by participating in this study.

Compensation: There will be no form of direct compensation in this study.

Confidentiality: Your name or any identifying information will not be captured anywhere in the study. Your responses and laboratory results will be stored safely at the university under lock and key by the principal investigator who is the only one who will have access to the information. The results of this study or any published work arising from this study will not bear your name or any other direct identifier.

Contacts: As a participant in this study, you have a right to contact the principal investigator, supervisors, or the TASH/St paul's Hospital/ ALERT /Addis Ababa Health Bureau/ AAERC/ aau Ethics and Research Committee for any concerns before, during and even after the research has been conducted.

Please feel free to ask any questions.

I now request you to sign the consent form attached.

አባሪ I የግል መረጃ ቅጽ (Information sheet Amharic Version)

የጥናቱ ርዕስ፡-

በተመረጡ በአዲስ አበባ ከተማ ውስጥ በሚገኙ ሆስፒታሎች በስኳር በሽታ ምክኒያት ከሚመጣ የእግር ቁስለት ታካሚዎች ቁስለቱን የሚያመጡ ባክቴሪያ አይነቶች መድሃኒቶችን የመላመድ ሂደት ላይ የሚከናወን የዳሰሳ ጥናት፡፡

የዋና መርማሪ ስም፡ በረከተአብ ብርሃኑ ስልክ፡ +251934538341, Email:Berhanubereketeab@gmail.com

የአማካሪ ስም እና አድራሻ፡- ካሱ ደስታ +251911107099/ዶ/ር አብዱረዛቅ አህመድ +251913696612

ዶ/ር ሙሉጌታ ፀጋዬ +251961232952

የጥናቱ መግቢያ፡በረከተአብ ብርሃኑ እባላለሁ፡፡ በአዲስ አበባ ዩኒቨርሲቲ የህክምና ፋኩልቲ በጤና ሳይንስ ኮሌጅ የላቦራቶሪ ሳይንስ የማስተርስ ዲግሪ ተማሪ ነኝ፡፡ ባሁኑ ሰዓት የስኳር በሽተኞች ላይ የእግር ቁስለት(ኢንፌክሽን) እንዲፈጠር የሚያደርጉትን ባክቴሪያዎች እና መድሃኒቶችን የመላመድ ሂደታቸውን ለማወቅ ጥናት እያካሄድኩ ነው፡፡

አላማ፡የዚህ ጥናት አላማ በየካቲት 12 ፤አለርት፤በዘውዲቱ፤ራስ ደስታ፤በሚኒሊክ፤ በቅዱስ ጳውሎስ እና በጥቁር አንበሳ ሆስፒታል የስኳር በሽተኞች የእግር ቁስለት ታካሚዎች መካከል ኢንፌክሽኑን ያመጣውን ባክቴሪያ አይነት እና መድሃኒቶችን የመላመድ ሂደት መለየት፡፡

የጥናቱ ተሳታፊው ሚና፡እርስዎ በጥናቱ ለመሳተፍ ፍቃደኛ ከሆኑ በኋላ ለመጠይቁ ይተባበሩናል፡፡ በተጨማሪም ከእግር ቁስሎት ላይ የሚወሰድ የመግል ናሙና ይሰጣሉ፡፡ በጥናቱ የሚሳተፉት ፈቃደኛ ከሆኑ ብቻ ነው፡፡ ስለዚህ መሳተፍ ከጀመሩ በኋላ ማቋረጥ ወይም መመለስ የማይፈልጉት ጥያቄ ካለ ይለፈኝ ማለት ሙሉ መብትዎ ነው፡፡ በጥናቱ መሳተፍ ወይም አለመሳተፍ በሚሰሩት ስራ ላይ ምንም ዓይነት ጥቅምም ሆነ ጉዳት አይኖረውም፡፡

የጥናቱ ጉዳት፡ ከቁስሉ ላይ የሚወሰደው የቁስሉ ላይ ያለውን ፈሳሽ ወይም መግል በመጥረግ ወይም በመርፌ በመውሰድ ሲሆን ናሙናው በሚወሰድበት ወቅት የተወሰነ የህመም ስሜት ይኖራል፡፡ ዳሩ ግን ምንም ዓይነት የከፋ ጉዳት አያደርስም፡፡

የዚህ ጥናት ጥቅም፡ ለተጠያቂው ቀጥታ የሆነ ጥቅም የለውም ነገር ግን ከጥናቱ በሚገኘው ውጤት መሰረት በማድረግ ህብረተሰቡን ለወደፊት ተጠቃሚ ያደርጋል።

ክፍያ፡ በጥናቱ የሚሳተፍ ምንም ዓይነት ክፍያ አይከፈልም ምንም ዓይነት የሚያገኙት ገንዘብም አይኖርም፡፡

ሚስጥር ጠባቂነት፡የሰጡት ማንኛውም መረጃ ሁሉ ሚስጥራዊነቱ የተጠበቀ ነው፡፡ ስም ሳይገለፅ መረጃ በመሰብሰብ ተጠያቂው የሚሰጠውን መረጃ ሚስጥር ይጠበቃል።

ለማነጋገር፡በዚህ ጥናት ተሳታፊ እንደ መሆኖት መጠን በጥናቱ ሂደትም ሆነ ጥናቱ ከተጠናቀቀ በኋላ የጥናቱን መርምሪውን፤ አማካርውን ወይም ደግሞ የሆስፒታሉንም ሆነ የአዲስ አበባ ዩኒቨርስቲን የስነምግባር እና የሪሰርች ኮሚቴ የማነጋገር መብት አለዎት፡፡ጥያቄዎን ለማቅረብ ነፃነት ይሰማዎት!!!በመቀጠልም አባሪ የተደረገውን የፈቃደኝነት መግለጫ ፎርም ይፈርሙ

Annex 2: Informed Consent form

I …… (Name of participant), being 18 years and above have been informed about the study and hereby do consent to voluntarily participate in this study. The nature of the study has been explained to me by the principal investigator and I have been given opportunity to ask questions concerning the study, which have been answered to my satisfaction. The benefits and risks of this study have been clearly explained to me and I am aware that I am free to withdraw from this study at any point and this will not jeopardize the care I receive at this hospital. I therefore give consent to be interviewed and that information from my file can also be used having understood the purpose of the study.

Signature……Date…….

If illiterate;

Name of independent literate witness……., date…….. signature……..

Researcher`s statement:

I ……. confirm that I have explained to the patient the purpose and nature of the study.

Signature…….Date……

Please feel free to use the contacts provided below;

Bereketeab Berhanu. Email address; BerhanuBereketeab@gmail.com. Tel; 0934538341

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Dr. Abdurezak Ahmed (Internist & Endocrinologist, Assistant professor), Addis Ababa University, Addis Ababa, Ethiopia

Dr. Mulugeta Tsegaye (MD, Internist), ALERT hospital, Addis Ababa,Ethiopia

TASH/ St. Paul's/ ALERT /Yekatit 12/Zewditu/Ras Desta and Minilik Memorial Hospitals / AAERC/University of Addis Ababa Ethics and Research Committee. Addis Ababa. Tel; +251 11 275 5170. AHRI's address; tel +(251)113483752 fax +(251)113211563

አባሪ II የተሳታፊውን ፈቃድ መግለጫ (Consent form Amharic version)

እኔ……እድሜዬ ከ 18 አመት በላይ ሲሆን በዚህ ጥናት ላይ ለመሳተፍ በፍላጎቴ መፍቀዴን መግለፅ እወዳለሁ፡፡ ስለጥናቱ አላማና ሂደቶች በደንብ ገለፃ ተደርጎልኛል፡፡ምንም አይነት ያልተገነዘብኩት ነገር እንድጠይቅ ተመቻችቶልኛል፡፡በመሆኑም ጥሩ ግንዛቤ ተፈጥሮልኛል፡፡ እንዲሁም ከጥናቱ የሚኖረውን ጥቅም እና ጉዳት አውቂያለሁ የመሳተፍ ፣አለመሳተፍ፣ ከጀመሩ በኋላ ማቋረጥ ወይም መመለስ የማልፈልገው ጥያቄ ካለ ይለፈኝ ማለት እንደሚቻል እና በዚህም ምክኒያት በሆስፒታሉ የማገኘው ግልጋሎት ላይም ምንም አይነት ተፅዕኖ እንደሌለው ተገንዝቢያለሁ፡፡ በተጨማሪም በጥናቱ ምንም አይነት ክፍያ እንደማይሰጠኝ እና እኔም እንደማልከፍል ተገልፆልኛል፡፡ የጥናቱ አላማ ግልፅ ስለሆነልኝ ለመሳተፍ ተስማምቻለሁ፡፡ ፊርማዬንም እንደሚከተለው አስቀምጫለሁ፡

የጥናቱ ተሣታፊ ፊርማ _____ ቀን _____

የተማሩ ካልሆኑ፡

ከጥናቱ ጋር ግንኙነት የሌለው ሰው (የምስክር)

ስም……ቀን……ፊርማ……

ፍቃደኛነትን የጠየቀ ሰው ፊርማ _____ ቀን _____

የዋና መራማሪው አድራሻ፤

በረከተአብ ብርሃኑ ፤ የሕክምና ላቦራቶሪ ቴክኖሎጂ ዲፓርትመንት፣ የጤና ሳይንስ ኮሌጅ፣ አዲስ አበባ ዩኒቨርሲቲ-አዲስ አበባ፣ ኢትዮጵያ

ኢ-ሜይል፣ Berhanubereketeab@gmail.com

ስልክ ፣ +251934538341

አማካሪ: ካሱ ደስታ(ረዳት ፕሮፌሰር): የህክምና ላቦራቶሪ ሳይንስ ዲፓርትመንት፤የጤና ሳይንስ ኮሌጅ፤የአዲስ አበባ ዩኒቨርስቲ ዶ/ር አብዱረዛቅ አህመድ +251913696612ዶ/ር ሙሉጌታ ፀጋዬ +251961232952

Annex 3. English version of the questionnaire

The title of this study is "Bacterial profile and Antimicrobial susceptibility pattern of common bacteria isolated from diabetic foot infections at selected public hospitals of Addis Ababa, Ethiopia.''

Interview

We are grateful for your agreement to participate in this study. Now we are going to have an interview with you and the interview is about general socio demographic characteristics and clinical data. All of the answers you provide in this study will be kept confidential. The information you give us is very essential for this study. Therefore we respectfully ask you to give us the right response.

Interviewer Name _____ Questionnaire Number _____

Date of interview_____

Questionnaire English Version

Socio-demographic information (Mark 'x' inside ☐ for your answer)		
101.	Study ID	
102.	Full name of participant	
103.	Sex	Male ☐ Female ☐
104.	Age (in years)	_____
105.	Address	Rural ☐ Urban ☐
106.	Marital status?	Single ☐ Married ☐ Widowed ☐ Divorced ☐
107.	Education Level?	Illiterate ☐ 9-12 ☐ 1-8 ☐ TVET diploma ☐ University degree & above ☐

108.	What is your occupational status?	Student ☐ Daily laborer ☐ Government employee ☐ Merchant ☐ Private enterprise employee ☐ Housewife ☐ No job ☐ Under age ☐ Farmer ☐
109.	Monthly Income (ETB)	≤500 ☐ 501-1000 ☐ ≥1001 ☐
2. CLINICAL CHARACTERISTICS (Mark 'x' inside for your answer)		
201.	Point of care	Chronic care ☐ Diabetes clinic ☐ Others_____
202.	Duration of diabetes: (months)	
203.	Duration of foot ulcer: (months)	
204.	Type of treatment (Antibiotic) Taken:	
205.	Duration Antibiotic taken	
3. Risk Factors (circle on the answers)		
301.	Do you have previous ulcer	1 Yes 2 No
302.	Do you have previous Amputation	1 Yes 2 No
303.	Do you have a Retinopathy	1 Yes 2 No

304.	If yes are you taking medicine	1 Yes 2 No
305.	Do you have a High Blood pressure	1 Yes 2 No
306.	If yes are you taking medicine	1 Yes 2 No
307.	Do you have renal disease	1 Yes 2 No
308.	If yes are you taking medicine	1 Yes 2 No
309.	Do you have peripheral neuropathy	1 Yes 2 No
310.	If yes 1 when started 2 Do you have follow up neuron Clinic	…… year(month 1 Yes 2 No)
311.	Do you have a PVD	1 Yes 2 No
312.	Do you have any dermatology Disease	1 yes 2 no
313.	what is your foot skin texture	1 Dry 2 Moist 3Cracked
314.	Do you always wear good supportive shoes	1Yes 2No
315.	Do you wash and dry your feet specially between the toes	1Yes 2No
316.	Do you check the color of the legs and feet if there is warmth, redness	1Yes 2No
317.	If you have pain do you see your Doctor	1Yes 2No
318.	If no, why reason	_____
319.	Do you have annual foot examination by professional	1Yes 2No

320.	Do you check fasting blood sugar	1Yes 2No
321.	If yes, how many times do you Check	1.every week 2.every two week 3.every month
322.	Have you ever received foot care education	1Yes 2No
323.	Do you perform regular exercise	1Yes 2No
324.	Do you avoid going bare foot outside indoor	1Yes 2No
325.	Do you check for foreign object in shoes before wearing them	1Yes 2No
326.	Do you Smoke?if so for how long?	1Yes 2No ……
327.	Do you drink alcohol?if so for how long?	1Yes 2No ……

Chart review

1. Type of DM…….

2. Any sign of infections……..

3. Laboratory parameters;-

- FBS……..
- HgbA1C……..
- BMI……..
- Total cholesterol……..
- Triglyceride…….
- LDL…….
- HDL……
- SGOT……
- SGPT……
- ALP……
- UREA……
- CREATININE……

አባሪ III. የጥናቱ መጠይቅ (Survey questionnaire Amharic version

የመረጃ መሰብሰቢያ መጠይቅ ፎርም

አዲስ አበባ ዩኒቨርሲቲ የላቦራቶሪ ት/ቤት በጥናቱ ተሳታፊ በሆኑ ግለሰቦች የሚሞላ ፎርም

የቃለመጠይቅ አድራጊው ስም _____የመጠይቅ ተ.ቁ _____

መጠይቅ የተወሰደበት ቀን _____

Questionnaire Amharic Version

1.የግለሰቡ አካላዊ ማህበራዊ እና ኢኮኖሚያዊ ዝርዝር		
ተ.ቁ	**ጥያቄ**	**መልስ**
101.	የመለያ ቁጥር	
102.	የግለሰቡ ስም	
103.	ጾታ	ወንድ ☐ ሴት ☐
104.	ዕድሜ	_____
105.	አድራሻ	ከተማ ☐ ገጠር ☐
106.	የጋብቻ ሁኔታ?	ያላገባ ☐ የትዳር ጓደኛ የሞተበት ☐ ያገባ ☐ የተፋቱ ☐
107.	የትምህርት ሁኔታ?	ያልተማረ ☐ 1-8 ☐ 9-12 ☐ TVET ዲፕሎማ ☐ ዩንቨርሲቲ ዲግሪ ና ከዛበለይ ☐
108.	የስራ ሁኔታ?	የግል ተቀጣሪ ☐ የመንግስት ተቀጣሪ ☐ ነጋዴ ☐ ገበሬ ☐ ተማሪ ☐ የግል ☐ የቤት እመቤት ☐ የቀን ሰርተኛ ስራ አጥ ☐ ሌላ _____
109.	የወር ገቢ	≤500 ☐ 501-1000 ☐ ≥1001 ☐
2.የምርመራው አጠቃላይ ሁኔታዎች		
201.	ተሳታፊው የሚታከምበት ክፍል?	የክሮኒክ ታካሚዎች ☐ የስኳር ታካሚዎች ክሊኒክ ☐ ሌላ……

202.	የስኳር ህመሙ የቆይታ ጊዜ? (በወራት)	
203.	የእግር ቁስለቱ ከጀመረ ምን ያህል ጊዜ ሆነው? (በወራት)	
204.	የወሰዱት የመድሃኒት አይነት ምንድን ነው?	
205.	መድሀኒቱን ለምን ያህል ጊዜ ወስደውታል?	
3.ተጓዳኝ አጋላጭ ሁኔታዎች		
301.	የእግር ቁስለት ከዚህ በፊት ነበረብዎ?	1 አው 2 አይደለም
302.	እግሮ በህክምና ምክንያት ተቆርጦ ነበር?	1 አው 2 አይደለም
303.	የዓይን በሽታ አለብዎ?	1 አው 2 አይደለም
304.	መልስህ/ሽ አዎ ከሆነ አስፈላጊውን ህክምና አድርገዋል?	1 አው 2 አይደለም
305.	የደም ግፊትህ/ሽ ከፍ ብሏል ?	1 አው 2 አይደለም
306.	መልስህ/ሽ አዎ ከሆነ መድሀኒት ይወስዳሉ?	1 አው 2 አይደለም
307.	የኩላሊት በሽታ አለብዎት?	1 አው 2 አይደለም
308.	መልስህ/ሽ አዎ ከሆነ መድሀኒት ይወስዳሉ?	1 አው 2 አይደለም
309.	በእጅ እና በእግርዎ የነርቭ ህመም አጋጥሞታል?	1 አው 2 አይደለም
310.	መልስሽ አዎን ከሆነ ለምን ያህል ጊዜ ይሆነዋል? የነርቭ ሀኪም አማክረዋል?	…… ጊዜ 1 አው 2 አይደለም
311.	የደም ዝውውር ጋር ተያያዥነት ያለው በሽታ አለብዎት?	1 አው 2 አይደለም

312.	የቆዳ በሽታ አለብዎ?	1 አው 2 አይደለም
313.	የቆዳ አይነትዎ ከየትኛው ይመደባል?	1 ደረቅ 2 ለስላሳ 3.የተሰነጣጠቀ
314.	ዘወትር ምቹ የሆነ ጫማ የለብሳሉ?	1 አው 2 አይደለም
315.	የውሃው የሙቀት ሁኔታ ተስማሚ በሆነ መልኩ እግሮን ይታጠባሉ?	1 አው 2 አይደለም
316.	በእግር ላይ የምታዩ ምልክቶችን ይከታተላሉ? ለምሳሌ የእግር መቅላት፤ሙቀት ወ.ዘ.ተ	1 አው 2 አይደለም
317.	የህመም ስሜቱ ሲኖር ከሀኪም ጋር ታይተዋል?	1 አው 2 አይደለም
318.	ካልታከሙ ለምን?	_____
319.	በየአመቱ የእግር ምርመራ ያከናውናሉ?	1 አው 2 አይደለም
320.	በየጊዜው የስኳር መጠኖን ይከታተላሉ?	1 አው 2 አይደለም
321.	መልሶ አዎ ከሆነ በምን ያህል ጊዜ ያሰራሉ?	1.በየሳምንቱ 2.በአስር አምስት ቀን 3.በወር
322.	ለእግሮ በቂ ጥንቃቄን እንዴት እንደሚወስዱ የጤና ትምህርት አግኝተው ያውቃሉ?	1 አው 2 አይደለም
323.	የሰውነት እንቅስቃሴ በበቂ ያከናውናሉ?	1 አው 2 አይደለም
324.	በባዶ እግር እንቅስቃሴ ያደርጋሉ?	1 አው 2 አይደለም
325.	ጫማውን ከመልበሶ በፊት ጫማው ውስጥ ምን ሊኖር እንደሚችል ያጣራሉ?	1 አው 2 አይደለም
326.	ሲጋራ ያጨሳሉ? ለምን ያክል ጊዜ?	1 አው 2 አይደለም
327.	አልኮል መጠጥ ይጠጣሉ? ለምን ያክል ጊዜ?	1 አው 2 አይደለም

ከታካሚው የህክምና ሰነድ ወይም ቻርት ላይ የሚወሰድ

1. የስኳሩ ዓይነት…….

2. የኢንፌክሽን ምልክት……..

3. የላቦራቶሪ ውጤት;-

- FBS……..
- HgbA1C……..
- BMI……..
- Total cholesterol……..
- Triglyceride…….
- LDL…….
- HDL……
- SGOT……
- SGPT……
- ALP……
- UREA……
- CREATININE……

Annex 4. Laboratory Sops

Gram stain

i. This is used to differentiate Gram positive (appears purple) and Gram negative (appears pink) bacteria. The following steps will be followed.

ii. Fixing the dried smear by passing over a flame three times.

iii. The fixed smear is covered with crystal violet for 30-60 seconds.

iv. The stain is rapidly washed with clean water.

v. All the water is tipped off and the smear covered with grams iodine.

vi. The iodine is washed with clean water.

vii. The smear is decolorized rapidly (in a few seconds) with acetone alcohol, then washed with clean water.

viii. The smear is then covered with neutral red stain for two minutes.

ix. The stain is then washed off with clean water.

x. The back of the slide is wiped clear and placed in a draining rack for the smear to air dry.

xi. The smear is then examined microscopically first with 40x objective to check the staining and see the distribution of materials and then in X 100 oil immersion objective to look for bacteria and cells morphology and arrangement.

Indole test

This is used to identify enterobacteria. Most strains of enterobacteria break down the amino acid tryptophan with the release of indole.

Principle:

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4(p) – dimethylamino-benzaldehyde. This reacts with the indole to produce a red coloured compound.

Method:

Using a sterile straight wire, 5ml of sterile medium is inoculated with test organism.

Incubate at 35-37^{o}C for up to 48hr.

Test for indole by adding 0.5ml of Kovac's reagent and shake gently.

Examine for red color in the surface layer with in 10 minutes.

Results:

Red surface layer…….Positive indole test

No red surface layer…….Negative indole test

Control

Positive control…….*Escherichia coli*

Negative control……...*Klebsiella Pneumoniae*

Motility

Spreading of turbidity throughout the medium is a positive proof.

Catalase test

Catalase test to differentiate staphylococci which produce the enzyme catalase from streptococci which are non catalase producing.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism will be tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer.

Procedure

1. Pour 2-3 ml of 3% hydrogen peroxide to a test tube

2. using a sterile wooden stick take the test organism and immerse into the hydrogen peroxide solution

3. Look for immediate bubbling

4. Interpretation:

Active bubbling . . . . . Positive catalase test

No bubbles . . . . . Negative catalase test

Controls

Positive coagulase control: Staphylococcus aureus

Negative coagulase control: Escherichia coli

Coagulase test

This test is used to identify S. aureus which produces the enzyme coagulase

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin.

Procedure

1. Place a drop of physiological saline on two separate slides

2. Emulsify the test organism in each of the drop to make thick suspension

3. Add one drop of plasma to one of the suspensions and mix gently. Look for clumping of the organism within 10 seconds

4. Clumping within 10 secs . . . . . S. aureus

No clumping within 10 secs . . . . . ….No bound coagulase

Controls

Positive coagulase control: Staphylococcus aureus

Negative coagulase control: Escherichia coli

If slide test is negative proceed to Tube test method

Tube test method (detects free coagulase)

Procedure

1. Take three small test tubes and label:

T _ Test organism (18–24 h broth culture)*

Pos _ Positive control (18–24 h S. aureus broth culture)*

Neg _ Negative control (sterile broth)*

2 Pipette 0.2 ml of plasma into each tube.

3 Add 0.8 ml of the test broth culture to tube T.

Add 0.8 ml of the S. aureus culture to the tube labeled ‘Pos’.

Add 0.8 ml of sterile broth to the tube labeled Neg’.

4. After mixing gently, incubate the three tubes at 35–37 _C. Examine for clotting after 1 hour

If no clotting has occurred, examine after 3 hours. If the test is still negative, leave the tube at room temperature overnight and examine again

Results

Clotting of tube contents or . . . . . S. aureus fibrin clot in tube

No clotting or fibrin clot . . . . . Negative test

Biochemical test for gram negative bacteria:- Identification of gram negative bacteria will be based on their test result with a series of biochemical tests.

Procedure

1. Prepare a suspension of the test organism with nutrient broth. 3-4 colonies of test organisms in 5 ml nutrient broth.

2. A loop full of the bacterial suspension is inoculated in to indole, citrate agar, KIA, lysine decarboxylase agar, manitol, urea agar and motility medium.

3. Incubate at 35-37 Oc for 18-24 hours

4. Look for color change (turbidity for motility) of the medium

5. Identify the test organism by considering the result of the biochemical tests

Oxidase test

This test is used to identify Pseudomonas spp.

Method

1) A piece of filter paper is placed in a petri dish and soaked with 2-3 drops of freshly prepared oxidase reagents.

2) Using a piece of stick or glass rod, a colony of the test organism is then be smeared on the filter paper.

3) Development of blue- purple colour within a few seconds indicate positive oxidase test.

Triple Sugar Iron (TSI) Test

- TSI Agar is a composit medium containing three fermentative sugars, lactose and sucrose in 1% concentrations and glucose in 0.1% concentration, Sodium thiosulfate and ferrous ammonium sulfate and Phenol red
- A yellow base indicates glucose fermentation
- A yellow base and slope indicates both glucose and lactose fermentation.
- Bubble in the medium indicate gas production from glucose
- Blackening of the medium indicate H_2S production

Urease test.

- This test is used to detect the enzyme urease, which breaks down urea into ammonia.
- Testing for urease enzyme activity is important in differentiating enterobacteria.
- *Brucella* and *Proteus* strains are strong urease producers.
- *Y. enterocolitica* also shows urease activity (weakly at 35-37^{0}C).

Principle

- The test organism is cultured in a medium which contains **urea** and the indicator **phenol red**.
- When the strain is urease-producing, the enzyme will break down the urea (by hydrolysis) to give **ammonia** and **carbon dioxide**.
- With the release of ammonia, the medium becomes alkaline as shown by a change in colour of the indicator to pink-red.

Method

Urease test using Christensen's (modified) urea broth

- Inoculate heavily the test organism in a bijou bottle containing 3 ml sterile Christensen's modified urea broth
- Incubate at 35-37^{0}C for 3-12 h (preferably in a water bath for a quicker result).
- Look for a pink colour in the medium.

Results

- Pink colour……..Positive urease test
- No pink colour…… Negative urease test

Citrate utilization test

The test detect the ability of an organism to use citrate as its only

source of carbon. This test is one of several techniques used

occasionally to assist in the identification of enteric bacteria.

Principle

- Some bacteria can obtain energy in a manner other than by the fermentation of carbohydrate by using citrate as source of carbon.

- The utilization of citrate by a test bacterium is detected in citrate medium by the production of alkaline by-products.
- The medium includes sodium citrate as the sole source of carbon and ammonium phosphate as the sole source of nitrogen.
- Bacteria that can use citrate can also extract nitrogen from the ammonium salt, with the production of ammonia (NH^+), leading to alkalinization of the medium.
- In the presence of the indicator Bromothymol blue the medium will be converted from green (at pH 6.0) to blue (at a pH above 7.6).

Material required

- Simmon's citrate medium/agar
- Inoculating loop

Method

1. Prepare slopes of the medium in bijou bottles as recommended by the manufacturer (store at 2-8 C)
2. Using a sterile straight wire, first streak the slope with a saline suspension of the test organism and then stab the butt.
3. Incubate at 35 ^{0}C for 48 hours
4. Look for a bright blue colour in the medium

Results

- Bright blue----------Positive citrate test
- No change in colour of medium ----------Negative citrate test

Controls

- Positive control ---------- *klebsiella pneumoniae*

- Negative control---------- *Escherichia coli*

Nitrate reduction test

- Nitrate broth is used for the test

Principle

- The test detects the ability of the organism to produce the enzyme nitrate reductase which reduses nitrate to nitrite

Method

- Growing the bacteria for 5 days at 37^0C in a broth containing 1% KNO_3
- Add 0.1 ml of the test reagent to the culture
- The test reagent consists of a mixture of equal volume of solutions of Sulphonilic acid and α-naphthylamine in 5 N acetic acid. Mixed just before use

Result

- Positive ---------Red colour developing within a few minutes
- Negative--------No colour change

Bacitracin test

This test is used to identify Streptococcus pyogenes.

Method

i. Bacitracin disk is placed on a culture plate inoculated with the organism and incubated at

35-37^{o}C overnight.

ii. A zone of inhibition around the disc is indicative of Streptococcus pyogenes.

Antimicrobial sensitivity testing- Disc diffusion method.

A disc of blotting paper is impregnated with a standard concentration of an

antimicrobial according to CSLI standards.

The disc is placed on a plate of susceptibility testing agar uniformly inoculated with the test organism.

The antimicrobial diffused from the disc into the medium and the growth of the test organism is inhibited at a distance from the disc that is related to the susceptibility of the organism.

Strains susceptible to the antimicrobial are inhibited at a distance from the disc whereas resistant strains had smaller zones of inhibition or grew up to the edge of the disc.

To ensure reproducibility and comparability of results, the modified Kirby-Bauer diffusion technique is used.

Modified Kirby-Bauer susceptibility testing technique

A sterile medium is prepared according to the manufacturer's instructions. The PH of the medium is set at 7.2-7.4.

The media is poured into a 90mm sterile petri dish to a depth of 4mm (about 25ml per plate). This is done on a level surface so that the depth of the medium is uniform. Note that if the media is too thin the inhibition zone will be falsely large and if too thick the zones will be falsely small.

Each new batch of agar is controlled using E. faecalis (ATCC 29212 or 33186) and cotrimoxazole disc.

The zone of inhibition should be 20mm or more in diameter.

The plates are stored at 2-8^{o}C in sealed plastic bags. For use the plates are dried with

their lids slightly raised in 35-37^{o}C incubator for about 30minutes.

About one hour before use, the working stock of the discs are allowed to warm to room temperature, protected from direct sunlight.

Method

1) Using a sterile wire loop, 3-5 well isolated colonies of similar appearance to the test organism are touched and emulsified in 3-4ml of sterile physiological saline or nutrient broth.

2) In a good light, the turbidity of the suspension is matched to the turbidity of the standard (the standard was mixed immediately before use). When comparing turbidities it is easier to view against a printed card or sheet of paper.

3) Using a sterile swab, a plate of Mueller Hinton agar is inoculated. Excess fluid is removed by rotating and pressing the swab against the side of the tube above the level of the suspension. The swab is streaked evenly over the surface of the medium in three directions, rotating the plate approximately 60^{o}C to ensure even distribution.

4) With the petri dish lid in place, 3-5 minutes are allowed (no longer than 15minutes) for the surface of the agar to dry.

5) Using sterile forceps, needle mounted in a holder, or multidisc dispenser, appropriate antimicrobial discs are placed evenly distributed on the inoculated plate. The discs should be 15mm from the edge of the plate and no closer than about 25mm from disc to disc. No more than eight discs are applied on each petri dish. Each disc are lightly pressed down to ensure its contact with the agar. It is not moved from one place.

6) Within 30minutes of applying the discs, the plates are inverted and incubated aerobically at 35^{o}C for 16-18 hours.

7) After overnight incubation, the control and the test plates are examined to ensure the growth is confluent or near confluent. Using a ruler on the underside of the plate, the diameter of each zone of inhibition is measured in mm. The endpoint of inhibition is where growth started.

Interpretation of zone sizes

Using the interpretative chart, the zones of each antimicrobial is interpreted reporting each organism as resistant, intermediate or susceptible.

Annex 5. Laboratory data collection Format

1.Identified micro-organisms

Gram positive bacteria

Bacteria	Isolate [tick where applicable]
Staphylococcus spp	
Streptococcus pyogenes	
Enterococcus	
Acinetobacterbaumanii	

Gram negative bacteria

Bacteria	Isolate [tick where applicable]
Pseudomonas aeruginosa	
Escherichia coli	
Klebsiellaspp	
Proteus spp	

Antibiotic sensitivity table

Bacteria……

Antibiotic	Resistant	Intermediate	Sensitive
Amoxicillin			
Clavulanate			
Cefuroxime			
Ceftriaxone			
Imipenem			
Ciprofloxacin			
Cefoxitin			
Cloxacillin			
Ceftazidime			

Declaration

I, the undersigned, declare that this M.Sc. thesis is my original work, has not been presented for a degree in this or any other university and that all sources of materials used for the thesis have been duly acknowledged.

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