Antibiotic Resistance in Aerobic Bacterial Isolates From Infected Diabetic Foot Ulcers in North Eastern Tanzania: An Urgent Call to Establish A Hospital Antimicrobial Stewardship Committee

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Research

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Abstract

Background.

Diabetic foot ulcers (DFU) is among major health problems which impact the socio economic burden globally. We aimed at assessing the susceptibility pattern of antimicrobials in DFU infections among patients admitted in the Surgical Department at Kilimanjaro Christian Medical Centre (KCMC).

Methods.

This descriptive cross-sectional study was conducted from September 2018 through March 2019. Pus swabs were collected on the first day of admission by deep wound swabbing after irrigation with normal saline solution. Kirby-Bauer method was done according to the Clinical and Laboratory Standard Institute (CLSI) guidelines.

Results.

Sixty diabetic ulcer patients had 62 bacterial isolates. Majority of the isolates were gram negative 49/62(79.03%). The most common isolate was Escherichia coli 15/62(24.19%) followed by Pseudomonas aeruginosa 14/62(22.58%), Proteus mirabilis 8/62(12.9%) and Staphylococcus aureus 5/62(8.06%). Klebsiella pneumoniae, Coagulase Negative Staphylococcus, Proteus Vulgaris, and Streptococcus pyogenes each contributed 4/62(6.25%) isolates. Of the 49/62(79.3%) gram negative isolates, 8/49(16.33%) were mono resistant, 30/49(61.22%) were multiresistant, and 11/49(22.45%) were susceptible. Of the multi-resistant isolates, E. coli 12/15(80.00%), and P aeruginosa 7/14(50.00%) were predominant. A total of 39/62(62.90%) isolates in patients contributed to poorer outcomes including loss of body part. Patients with ulcers infected by P aeruginosa 11/39 (28.21%) had the highest number of surgical removal of body parts followed by E. coli 8/39(20.51%). Gram negative bacteria were highly susceptible to amikacin 91.18%, meropenem 93.33% and imipenem 95.24%. Isolates susceptibility to ceftriaxone was 32%.

Conclusions.

Amikacin, meropenem and imipenem can be safely used as broad-spectrum antimicrobials in DFU. The Standard of care remains culture and sensitivity of isolated microorganisms in combating diabetic foot ulcers infections.

Background

Diabetic patients with diabetic foot infections cause significant health problems as they reduce quality of life, lead to amputations and are associated with increased cost of health services [1 –3]. This is exacerbated by the presence of resistant bacteria to antimicrobial agents (AMR). Thus, antibiotic resistance is considered a serious problem in medical-surgical care set-ups[4]. Diabetic patients are considered a high-risk group for development of surgical site infection as they are mostly immune suppressed [5, 6]. Prompt actions to reduce antibiotic resistance development are needed to protect the current antibiotics that are still working[7].

It is estimated that approximately 700,000 people die each year due to drug resistant pathogens. Projections show that AMR pathogens will cause about 10 million deaths each year by 2050[7]. In many cases AMR basically reflects irrational use of antibiotics with ultimate increase in selection pressure favoring emergence of drug resistant bacteria[8]. The effects of AMR on patients and health care systems are prolonged hospital stay, need for institutional care, high treatment costs and poor functionality[8, 9]. Furthermore, in the context of diabetes AMR leads to disarticulation or amputation and high both short- and long-term morbidities[10]. Besides these, AMR has been shown to be associated with significant distress to the patient and the family[11, 12].

It is important that empirical antibiotic prescription be guided by local susceptibility patterns data to reduce inappropriate antimicrobial use[13]. No data exists in Sub Saharan African countries, on the epidemiology of AMR among diabetic patients with DFU. This is due to lack of standardized diagnosis, absence of effective surveillance system and uniform notification system in sub-Saharan African countries[14 –16]. Tanzanian National Action Plan on Antimicrobial Resistance addressing actions in combat AMR was launched in August 2017[17]. It aims, among other things, to insist on rational use of antibiotics by targeted prescribing. However, local susceptibility patterns on diabetic wounds remain undocumented leading to empiric antibiotic prescribing. Local susceptibility data may aid in controlling irrational use of antimicrobials and mitigate AMR in Tanzania. Earlier studies have put forward Staphylococcus aureus, Klebsiella pneumonia and Escherichia coli to be the most prominent isolated bacteria among DFU patients [18, 19].

One study showed the majority of resistant strains of methicillin resistant Staphylococcus aureus of 35%, K. pneumoniae and E.coli resistance rates of 38.5% and 29.3% for ceftriaxone respectively; and that majority of the invasive infections were gram negative bacteria[20]. Litle is known on antibiotic resistance among diabetic patients with infected foot ulcer in Tanzania. Based on limited available antimicrobial resistance data on diabetic ulcers in resource limited settings it is obvious that the empiric management of diabetic foot ulcer may be ineffective and more costly to an individual and entire health system. Therefore, we aimed at identifying bacterial aetologies, their sensitivity patterns on the commonly prescribed antibiotics among patients with infected DFU in surgical wards of KCMC, a tertiary and a University teaching hospital in Moshi municipality, North eastern Tanzania.

Materials And Methods

Study settings, design and population
The study was done in Kilimanjaro Christian Medical Centre (KCMC). KCMC is a consultant, teaching and referral hospital serving a population of over 11 million people from northern and central regions of Tanzania (http://www.kcmc.ac.tz/). This was a hospital based descriptive cross-sectional study conducted among diabetic foot patients admitted in the General Surgery Department at KCMC for a period of 6 months from September 2018 through March 2019.

Clinical laboratory procedures

Pus specimens from in-patients were collected on the first day of hospital admission by deep wound swabbing after the wounds were irrigated with normal saline solution. The specimens were submitted to the KCMC clinical laboratory in Stuart Transport medium for testing. The specimens were aerobically subjected to culture on Blood Agar and Mac-Conkey Agar plates. Bacterial isolates were Gram stained. Susceptibility tests for the isolated pathogens were performed by Disc Diffusion (Kirby-Bauer) method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Patients antibiotic regimes were then tailored as per their pus swab culture and sensitivity results.

Clinical management of patients

Enrolment in the study required the patient to be above 18 years, be diagnosed with diabetes and an infected ulcer below the malleolus. A clinical diagnosis of an infected ulcer was based upon signs of purulent discharge, pain, erythema, warmth and induration. Prior pus swab culture and sensitivity results, patients were instituted on broad spectrum antibiotics and switched accordingly post sensitivity results. Daily wound dressing with normal saline was done with closed method of wound dressing with gauze. Serial dressing with sharp dedridement was done to remove sloughs with dead tissues. Non healing ulcers with deep tissues destruction and infection, involved limbs underwent either transtibial or femoral amputation.

Data management

Data were abstracted from patients’ files using a structured questionnaire, transferred and processed using STATA version 14.0 (Stata Corp, College Station, TX, USA). Data checking for inconsistency or missing values, formatting variables like dates/time, variables transformation, generating, recoding variables, duplicate entries or any unusual values (outliers) were identified and removed prior to analysis. Data analysis was based on complete case analysis.

Data analysis

Data were descriptively analysed. Categorical variables were summarized as frequencies/proportions.

Ethical Considerations

Ethical Clearance was obtained from Kilimanjaro Christian Medical University College Research and Ethical Committee (CREC) certificate number 2366. Confidentiality was ensured in that no personal identifying information was written in the data capture or database. Written informed consent to participate in the study was obtained from study participants. Participants were clearly made to understand that no participation in the study would in no way jeopardize clinical management in the ward.

Results

Sixty diabetic ulcer patients had 62 bacterial isolates from 12 bacterial species. Majority were gram negative, contributing to almost two thirds 49(79.03%) of all isolates. The most common isolate was *Escherichia coli* 15(24.19%) followed by *Pseudomonas aeruginosa* 14(22.58%), *Proteus mirabilis* 8(12.9%) and *Staphylococcus aureus* 5(8.06%). *Klebsiella pneumoniae*, *Coagulase Negative Staphylococcus*, *Proteus Vulgaris*, and *Streptococcus pyogenes* each contributed 4(6.25%) isolates. The rest of isolates including *Acinetobactor spp*, *Citrobacter spp*, *Morganella morgani*, non-fermenting gram-negative bacilli each contributed 1(1.61%) isolate. (figure 1).

Of the 13(20.97%) isolates of gram positive, 2(15.38%) were mono-resistant, 3(23.08%) were multidrug resistant to the antimicrobials tested, and 8(61.54%) were susceptible. Of the 49(79.3%) gram negative isolates, 8(16.33%) were mono-resistant, 30(61.22%) were multidrug resistant and 11(22.45%) were susceptible. (table 1).

| TABLE 1: Gram staining status and extent of resistance |
|--------------------------------------|-----------------|-----------------|-----------------|
| Gram status                         | Drug resistance          |                  |                  |
|                                      | §Mono-resistant (n(%))   | multidrug resistant (n(%) | Susceptible (n(%)) |
| Gram negative                       | 8(16.33)                  | 30(61.22)         | 11(22.45)         |
| Gram positive                       | 2(15.38)                  | 3(23.08)          | 8(61.54)          |
| No Growth                           | -                           | -                   | 6(100.00)         |
| Total                               | 10(14.71)                 | 33(48.53)          | 25(36.76)         |

§ Mono-resistance refers to bacteria spp with resistance to only one of the tested antibiotics.

Of the 60 patients in the study, 47(78.33%) had single bacterial isolate, 7(11.67%) had multiple bacterial isolates and 6(13.04%) with no bacterial growth. (figure 2.)
Considering individual isolates with multiple antibiotic resistance, *Escherichia coli* 12/15 (80.00%) and *Pseudomonas aeruginosa* 7/14 (50.00%) had the highest levels of multiple antibiotic resistance to the antimicrobials tested. More than half 33/62 (53.23%) of the total isolates had multiple antibiotic resistance. (table 2)

**TABLE 2:** Microbial isolates compared by resistance to a single or multiple drugs.

| Isolate               | §Monoresistant (n%) | Multidrug resistant (n%) | Susceptible (n%) |
|----------------------|--------------------|--------------------------|-----------------|
| Other Species        | 1 (8.33)           | 8 (66.67)                | 3 (25.00)       |
| *Escherichia coli*   | 3 (20.00)          | 12 (80.00)               | -               |
| *Proteus species*    | 3 (25.00)          | 5 (41.67)                | 4 (33.33)       |
| *Pseudomonas aeruginosa* | 2 (14.29)        | 7 (50.00)                | 5 (35.71)       |
| *Staphylococcus species* | 1 (11.11)        | 1 (11.11)                | 7 (77.78)       |
| Sterile Growths      | -                  | -                        | 6 (100.00)      |
| **Total = 62**       | 10 (16.13)         | 33 (53.23)               | 25 (36.76)      |

§ Mono-resistance refers to bacteria spp with resistance to only one of the tested antibiotics.

A total of 15 isolates were tested against meropenem where 14 (93.33%) were sensitive. For imipenem 21 isolates were tested whereby 20 (95.23%) were sensitive. Isolates tested against amikacin were 34 whereby 27 (79.41%) were susceptible. With regard to gentamicin 40 isolates were tested where 22 (55.00%) were susceptible. Ciprofloxacin was tested against 34 isolates whereby 16 (47.06%) were sensitive. Ceftriaxone, a common antibiotic in used in the ward, was tested against 25 isolates, whereby 8 (32.00%) were sensitive and 17 (68.00%) were resistant. A combination of Amoxicillin and Clavulanic acid was tested against 29 isolates whereby 8 (27.56%) were sensitive. (table 3)

**TABLE 3:** Aetiologies and the sensitivity patterns of bacterial clinical isolates

| Clinical isolate       | Staphylococcus species | *Escherichia coli* | *Proteus species* | *Pseudomonas aeruginosa* | Others |
|------------------------|------------------------|---------------------|---------------------|--------------------------|--------|
| Antibiotic             | S % n %                | R % n %             | S % n %             | R % n %                  | S % n % |
| Ceftriaxone            | 1 11.1 1 5.6           | 2 22.2 9 50 5 55.6 5 27.8 1 11.1 2 11.1 0 0 1 5.6 |
| Ciprofloxacin          | 3 15.8 0 0             | 4 21.1 5 27.8 5 26.3 2 11.1 5 26.3 6 33.3 2 10.5 5 27.8 |
| Meropenem              | 5 33.3 6 40            | 2 13.3 2 13.3       |
| Imipenem               | 6 28.6 5 23.8          | 5 23.8              |
| Amikacin               | 7 25.9 2 28.6 6 22.2 0 0 9 33.3 3 42.9 5 18.5 2 28.6 |
| Gentamycin             | 3 12 1 5.3             | 7 28 6 31.6 7 28 3 15.8 5 20 6 31.6 3 12 3 15.8 |
| Amoxicillin-clavulanic acid | 2 20 0 0 1 10 10 47.6 5 50 6 26.8 1 10 1 4.8 1 10 4 19 |

§ R refers to Resistance and S sensitivity of the bacterial spps versus tested antibiotics.

Thirty-nine out of sixty-two (62.90%) isolates contributed to poorer outcomes including loss of a body part. Patients with ulcers infected by *Pseudomonas aeruginosa* 11/39 (28.21%) had the highest number of surgical removal of body parts followed by *Escherichia coli* 8/39 (20.51%). (table 4)

**TABLE 4:** Bacterial isolates and treatment outcomes among DFU patients
of antimicrobials, through antimicrobial stewardship programs, to mitigate escalation of antimicrobial resistance.

In this study, more than a half of the isolates 17/25 (68%) were resistant to ceftriaxone. This is unarguably a high resistance level to a third-generation cephalosporin class of antibiotics. Ceftriaxone has been, over time, excessively and inappropriately prescribed in hospital settings in Tanzanian hospitals[28]. It is the non-chalant use of this important class of antibiotics that has resulted to such a high level of bacterial resistance against ceftriaxone. Our data have shown, however, that the next higher level of antibiotic use, carbapenems, are still very useful where the bacterial resistance against carbapenems were very low. Imipenem resistance was 1/21(4.76%) and meropenem 1/15(6.67%). Carbapenem use in Tanzania is still low currently.

From 60 patients with infected DFU in this study, a single bacterial isolate was isolated from 47(78.33%) patients, 6(10.00%) had two isolates, 1(1.67%) had three isolates and 6(13.04%) had no bacterial growth. Although we did not perform regression analyses on the association between multiple isolates and treatment outcome due to low numbers, our data indicates adverse outcome with multiple isolates. For instance, of all patients with multiple isolates 4/7 (57.14 %) ended up with some form of major limb amputation. This poor prognosis may be explained by the fact that diabetes is an immune suppressive disease and multiple bacterial infection indicates poor glycemic control[23].

Sixty patients were recruited in the study. Thirty-five (58.33%) were males. Total number of isolates was 62. The most prevalent isolates were E. coli 15/62(24.2%) followed by Pseudomonas aeruginosa 14/62(22.6%) Proteus spp 12/62(19.4%) and then Staphylococcus spp 9/62(14.52%). Other isolates occurred in small numbers such as Acinetobacter spp 1/62(1.61%), Citrobacter spp 1/62(1.61%), Klebsiella pneumonia 4/62(6.45%), Streptococcus spp 4/62(6.45%), Morganella morgan 1/62(1.61%) and non-fermenting gram negative bacilli 1/62(1.61%) whose prevalence was collectively 12/62(19.3%).

Our results are similar to a study in India where the most common gram-positive cocci in order of frequency were Staphylococcus aureus (17%), Streptococcus spp, (6%) and Enterococci spp, (5.0%). Escherichia coli (20%) was the predominant isolate followed by Pseudomonas spp, (18%), Klebsiella spp, (10%), Proteus spp, (6.0%) and Acinetobacter spp, (3%) in gram negative bacilli. However, the Indian study had Coagulase Negative Staphylococcus (CONS) prevalence of 12% which is double our prevalence. This discrepancy may be due to the fact that the Indian study had 148 isolates which is more than twice the number of our isolates[21]. The source of infection, use of antibiotic drug for treatment, sample collection method, and different types of infection can influence pathogen diversity in DFI[22].

Majority of the bacterial isolates were gram negative and were multi-resistant. Of the 49/62(79.3%) gram negative isolates, 8(16.33%) were monoresistant, 30(61.22%) were multi-resistant, and 11(22.45%) were susceptible. Of the multi-resistant isolates, Escherichia coli 12/15(80.00%), and Pseudomonas aeruginosa 7/14(50.00%) were predominant. Of the 13/62(20.97%) Gram positive isolates, 2(15.38%) were monoresistant, 3(23.08%) were multi resistant, and 8/13(61.54%) were susceptible. Studies have identified factors responsible for multi-resistance to be frequent hospitalization, recent use of broad-spectrum antibiotics, inadequate surgical source reduction, chronic wounds, irrational use of antibiotics, and the transfer of resistance genes by transport means[22]. A high level of multi drug resistance could be due to the fact that in a tertiary care hospital there is a widespread usage of broadspectrum antibiotics leading to selective survival advantage of pathogens, a phenomenon called antibiotic selection pressure[27].

In this study, more than a half of the isolates 17/25 (68%) were resistant to ceftriaxone. This is unarguably a high resistance level to a third-generation cephalosporin class of antibiotics. Ceftriaxone has been, over time, excessively and inappropriately prescribed in hospital settings in Tanzanian hospitals[28]. It is the non-chalant use of this important class of antibiotics that has resulted to such a high level of bacterial resistance against ceftriaxone. Our data have shown, however, that the next higher level of antibiotic use, carbapenems, are still very useful where the bacterial resistance against carbapenems were very low. Imipenem resistance was 1/21(4.76%) and meropenem 1/15(6.67%). Carbapenem use in Tanzania is still low currently. However, with such increasing trend of ceftriaxone resistance, carbapenem use is likely to occur. In the face of lack of new molecules from pharmaceutical companies in the last three decades, we risk reverting to a pre-antibiotic era after running out of therapeutic options[4, 29]. To reverse this trend, we need to practice a judicial use of antibiotics by promoting hospital antimicrobial stewardship programs[30–32]. Hospital antimicrobial stewardship programs cannot be over emphasized to mitigate escalation of antimicrobial resistance[33]. A similar observation was done in Mwanza, Tanzania where isolates showed high resistance to commonly used antibiotics (such as ampicillin, augmentin, cotrimoxazole, tetracycline, penicillin, gentamicin, erythromycin, oxacillin) except for meropenem and imipenem, which were both 100% sensitive[34]. The low resistance to carbapenems is similarly observed in India where sensitivity to imipenem, meropenem were high; imipenem (89%) and meropenem (84%)[22]. An important decision is not to switch to carbapenems but make a judicial use of antimicrobials, through antimicrobial stewardship programs, to mitigate escalation of antimicrobial resistance.

| Isolate                  | Surgical removal of body part | Debridement |
|-------------------------|--------------------------------|-------------|
|                         | N  | %   | N  | %   |
| Staphylococcus species  | 3  | 33.3| 6  | 66.7|
| Escherichia coli        | 8  | 53.3| 7  | 46.7|
| Proteus species         | 8  | 66.7| 4  | 33.3|
| Pseudomonas aeruginosa  | 11 | 78.6| 3  | 21.4|
| Others                  | 9  | 75.0| 3  | 25.0|

§ Any loss of limb or part of it due to a patient undergoing either some form of major limb amputation (above or below knee amputation) or disarticulation of digits.

### Discussion

| Procedure                  | N | %   |
|----------------------------|---|-----|
| Surgical removal of limb   | 21| 21.4|
| Debridement                | 46| 75.0|
| Amputation                 | 7 | 11.1|

From 60 patients with infected DFU in this study, a single bacterial isolate was isolated from 47(78.33%) patients, 6(10.00%) had two isolates, 1(1.67%) had three isolates and 6(13.04%) had no bacterial growth. Although we did not perform regression analyses on the association between multiple isolates and treatment outcome due to low numbers, our data indicates adverse outcome with multiple isolates. For instance, of all patients with multiple isolates 4/7 (57.14 %) ended up with some form of major limb amputation. This poor prognosis may be explained by the fact that diabetes is an immune suppressive disease and multiple bacterial infection indicates poor glycemic control[23]. We observed a similar finding in Egypt where a study showed predominance of single bacterial isolate in 52% of the cultures, 40% with mixed infections and 8% with sterile growth. [24]. However, a Nigerian study showed a different observation where there was a predominance of multiple bacterial infections of approximately 71.2%, which is higher than our findings[25]. There are, however, situations where single and multiple isolates occur in the same proportions. This was the case in India where the proportion of multiple isolates was 48/108(44.4%), single isolates was 48/108(44.4%), and no growth in 12/108(11.1%)[26].
With special reference to *Pseudomonas aeruginosa*, in this study, 14/62 (22.58%) were isolated and of the tested isolates 7/14 (50.00%) were multi-resistant to the tested antibiotics and 2/14 (14.29%) were monoresistant. As high as 11/14 (78.57%) of patients from whom *Pseudomonas aeruginosa* were isolated ended up having a major limb amputation. Two Indian studies show that pseudomonal control requires "reserve" antibiotics that are not routinely available in hospital settings in Tanzania as essential drug list. One study showed that 15 (83.3%) *Pseudomonas aeruginosa* strains were susceptible to cefotaxime with a resistance rate of (16.6%) [35]. Another showed that *Pseudomonas* culture isolates were sensitive to amikacin (90%), imipenem (72%), meropenem (70%), and piperacillin-tazobactam combination (74%) [22].

With regard to some form of major limb amputation or surgical removal of body parts, *Pseudomonas aeruginosa* had the highest number of isolates 11/14 (78.57%) followed by *Escherichia coli* 7/15 (46.67%). A major problem in *Pseudomonas aeruginosa* infection may be that this pathogen exhibits a high degree of resistance to a broad spectrum of antibiotics because of its ability (intrinsic) to produce β-lactamases, efflux pumps, outer membrane modification, and biofilm lifestyle thus making it a dangerous and dreaded pathogen. Most infections with *Pseudomonas* spp occur in compromised hosts [35]. The high rate of amputations among patients from whom *Pseudomonas aeruginosa* was isolated might be due to its ability to cause severe tissue damage in diabetics, its inherent resistance mechanism, referred to as intrinsic resistance and its multiplicity in resistance mechanisms [35]. Our data indicate how difficult it is to treat a diabetic patient with an ulcer infected by *Pseudomonas*. Being nosocomially acquired, hospital infection prevention and control (IPC) is a mandatory component of mitigation of antimicrobial resistance [36]. This study commands some strengths in showing an adequate description of the microbiological isolates with reference to treatment outcome. It describes a clinical picture of the different spectrum of bacterial infection complications in relation to the type of organism isolated from a diabetic foot ulcer.

**Limitations**

This study had some limitations in that data was collected for only six months duration of which only 60 patients could be recruited into the study. Diabetic foot ulcer infections admissions are about 7 patients per month. This fact rendered us only able to perform descriptive analysis of the data. Anaerobic and fungal microbiological cultures were not performed in this study. The method used in collecting the specimens was only deep pus swabs. Patients who were enrolled in this study at KCMC as a Tertiary Care Centre might have been exposed to prior antibiotic treatment at primary/peripheral centers.

**Conclusion**

The most common isolates were gram negatives aerobes, with *Escherichia coli* and *Pseudomonas aeruginosa* being the most isolated species. *Staphylococcus aureus* was the most isolated gram-positive species. More than three quarters of patients with *P. aeruginosa* infection had some form of major limb amputation. For gram negative spp the most effective antibiotic was amikacin, imipenem and meropenem. Ciprofloxacin, sulfamethoxazole/trimethoprim and gentamicin showed limited effectiveness. Ceftriaxone, a commonly used antibiotic in our settings, showed poor effectiveness. All cases of DFU infection should therefore be subjected to culture and antibiogram sensitivity testing for targeted infection management. In situations where culture and sensitivity pattern data are not available amikacin, imipenem and meropenem can be given as broad-spectrum antibiotics prior to availability of culture and sensitivity results.

These antibiotics have been shown to be highly effective against gram negative aerobes which are the predominant isolates in DFU infections. A larger scale study on DFU should be conducted over a longer duration of time for analytical analysis. Different methods of specimen collection involving pus swabs, tissues biopsy to give a wider picture on the spectrum of bacterial isolates in diabetic foot ulcer are warranted.

**Declarations**

**Data Availability**

The data used to support this study are available from the first author upon request

**Conflict of Interest**

The authors declare that they have no conflicts of interest

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**Authors’ Contributions**

This work was carried out in collaboration between all authors. Ahmed Shabhay wrote the research proposal, dissertation thesis and collected the data. Samweli Chugulu, Jeff Baal, Kondo Chilonga and David Msuya reviewed the research proposal and dissertation thesis. Ahmed Shabhay, Pius Horumpende, Andrew Mganga, Martin Mujuni and Edna Joy Munisi did data analysis and Interpretation. Ahmed Shabhay and Pius Horumpende prepared the first draft of the manuscript. Samweli Chugulu, Kondo Chilonga, David Msuya, Martin Mujuni, Edna Joy Munisi, Zarina Shabhay, Stephen Mshana and Jaffu O Chilongola reviewed and contributed to the final version of the manuscript. All authors have read and approved the final manuscript.

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**Figures**

![Pie chart showing distribution of clinical isolates](image)

**Figure 1**

The distribution of clinical isolates(N=62)
Figure 2

Number of patients with single or multiple microbial isolates. (N=60)