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# Molecular Characterization of Multi Drug Resistant Bacteria Isolated from Diabetic Foot Infections in Jabir Abolez Diabetic Foot Center - Khartoum - Sudan

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## Abstract

**Background:** Diabetic foot ulcers (DFU) are among major health problems which impact the socio-economic burden globally. Multi-drug resistance has become a major problem for the treatment of pathogenic bacterial infections.

**Objective:** The present study was aimed to determine the molecular characterization of multi drug resistance bacteria isolated from diabetic foot Infections patients with septic foot infection attending Jaber Abolez Diabetic Center from the 1st of February till the 31st of July 2021.

**Methods:** This descriptive cross-sectional study. Wound swabs were collected from each wound after the wound had been cleansed and debrided. Antibiotic susceptibility tests were performed by Kirby-Bauer disc diffusion and B-lactamase genes were detected used PCR.

**Results:** The most common isolated bacteria were *Proteus mirabilis* (80.0% of cases) followed by *Enterococcus faecalis* (63% of cases) and *Staphylococcus aureus* (36% of cases). The isolated bacteria were 43% aerobic Gram negative, 21% aerobic gram positive, 19% anaerobic gram positive and anaerobic gram negative. Most of the isolated bacteria were resistant to multiple antibiotics especially for ciprofloxacin (100% of the isolated bacteria) and cephalosporins and ampicillin (98% of the isolated bacteria). The lowest cases of resistance were to meropenem. The commonest multi-drug resistance gram negatives of the B-lactamase genes using PCR was extended spectrum (ESBL) bla SHV which detected in 83% of isolated gram-negative bacteria followed by extended spectrum (ESBL) bla *CTXM-1* and Carbapenemase gene *bla NDM*; 66% and 61% respectively.

**Conclusion:** Multidrug-resistant facultative anaerobic bacteria are overrepresented as agents of DFU. High rates of resistance toward most of the tested antibiotics were reported. Gram-negative organisms expressed high rates of extended spectrum B-lactamase genes. BlaSHV was the most detected gene.

***Keywords:*** *Bacteria; Diabetic Foot; Multi Drug Resistance; PCR; β-Lactamase; Sudan*

## Introduction

Diabetic foot infections (DFIs) constitute a major clinical and financial burden to the diabetic patients. DFIs are one of the most feared complications of diabetes that can progress rapidly to irreversible septic gangrene necessitating amputation of the foot [1-3]. Patients with diabetes are 25 times more likely to lose a leg than those without diabetes, and up to 70% of all leg amputations occur in people with diabetes [4,5].

Microbiological studies have indicated the polymicrobial nature of DFIs, the most frequently identified isolates being aerobics isolates [2,3,6]. Worldwide, several studies have been conducted with respect to the bacteriology and antibiotic sensitivity patterns of DSF concluded that aerobes are considered causing the most illnesses and pathogenicity. However, chronic wounds develop more complex colonizing flora, like, *Enterococcus* spp, *Escherichia coli, Pseudomonas aeruginosa, Proteus mirabilis* and *Klebsiella pneumoniae* [7-9]. The most common anaerobic isolates are Pepto *streptococcus* spp, *Bacteroides fragilis* has also been reported in several studies as the most abundant anaerobic bacteria in DFIs [10,11] and *Clostridium* spp. [12,13].

Antibiotic resistance is considered to be a major threat in the treatment of DFIs. Carriage of MDR microorganisms is mostly due to inappropriate antibiotic treatment., reduced antibiotic concentration in the tissue, inadequate selection and abuse of antibiotics, chronic course of the wound, reduced effect of antibiotics in the wound environment and frequent hospital admission of DFU patients, where they are likely to be exposed to MDR organisms [8,14,15].

Incidence of antibiotics resistance is becoming a serious problem, especially with the DSFs patients where few systematic studies had been made to unravel the occurrence of MDR bacteria and/or the genetic basis of resistance in these bacteria [7]. Infection in DSFs with MDR bacteria is known to increase the duration of hospital stay cost of management as well as morbidity and mortality. Henceforth, early diagnosis of microbial infections and appropriate antibiotic therapy are needed to avoid further complications Extended-spectrum beta-lactamases (ESBL) are enzymes that confer resistance to most beta-lactam antibiotics, including penicillin's, cephalosporins and the monobactam aztreonam. Infections with ESBL-producing organisms have been associated with poor outcomes.

Community and hospital-acquired ESBL-producing Enterobacteriaceae are prevalent worldwide. Reliable identification of ESBL-producing organisms in clinical laboratories can be challenging, so their prevalence is likely underestimated. Carbapenems are the best antimicrobial agent for infections caused by such organisms [16].

In the present study, an attempt was made to determine the isolation of aerobic and anaerobic bacteria from Sudanese patients with diabetic foot infections and to assess their *in vitro* susceptibility to the commonly used antibiotics, as well as to study their genetic resistance markers of the commonest isolates.

## Materials and Methods

### Study design

This is a descriptive cross-sectional study was conducted during the period from 1st of January to 31st of July, 2021 at the Jaber Abolez Diabetic Center (JADC), which is a specialized center in Sudan for the treatment and follow up of diabetic patients with septic foot infections and central laboratory ministry of higher education and scientific research. All septic foot infection patient's attending JADC for wound dressing and culture inquiries recruited to the study after the approval of them. Diabetic patients with sterile foot infections were excluded from this study. A total of 50 patients with DFIs (37 were males and 13 were females) with age group ranging from 60 to 80 years old. All patients were informed of the purpose of the study and their consent was sought.

### Sample collection and processing

Two specimens were obtained from the foot ulcers using, commercially purchased swabs one for Gram stain and the other for aerobic and anaerobic bacteria and transported to the microbiology laboratory immediately. The sample analysis examined in Central Laboratory, Ministry of Higher Education and Scientific Research Khartoum.

### Data collection

A structured questionnaire and patient clinical sheet were used; demographic data and other Data (clinical symptoms, previous antibiotic, duration of antibiotic used). Verbal consent was obtained from each patient enrolled in this study.

### Identification of the isolated bacteria

Clinical samples were inoculated on blood agar medium and MacConkey agar medium; Incubation were done aerobically and anaerobically. For aerobic bacteria, they were cultured on blood agar plates, MacConkey medium and in Robertson's Cooked Meat (RCM) medium. The media were incubated at 37°C, overnight. The broth culture for further subcultures onto the same above-mentioned solid media after overnight incubation and the plates were incubated aerobically. The specimens were also cultured anaerobically on culture plates incubated in anaerobic Gas pack jars. The Gas pack jars opened after 48 - 72 hours for inspection of plates. The inoculated RCM broths were incubated for seven days and subcultures were done on to blood agar if any additional bacterial morph types were noted on Gram stain from the broth. Identification of organisms was done using standard conventional biochemical tests (Kligler Iron agar: slant/Acid, butt/Acid, $H_2S$ production/-, Gas/+; Motility test/motile; Indole/+, Urease/-; Citrate/-) [18].

### Antimicrobial susceptibility testing

All identified gram-negative bacteria were tested for their antimicrobial susceptibilities by disc diffusion technique according to the Clinical Laboratory Standards Institute (CLSI) guidelines. The antibiotic discs were used: Meropenem (10 µg), Amikacin (30 µg), Gentamycin (10 µg), Cefepime (30 µg), Cefpodoxime (10 µg), ceftriaxone (30 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg) and Colistin (10 µg) (Himedia Co. India).

Several colonies of tested organism were emulsified in small volume of sterile normal saline to make a fine suspension. The turbidity of the suspension was matched against 0.5 McFarland standards. A sterile cotton swab was dipped into the suspension and was rotated firmly several times against the upper side wall of the tube to exclude the excess fluid. Then the entire agar surface of Mueller-Hinton agar plates was streaked three times, the plates were turned 60 degrees between streaking to obtain even inoculums. The lid of the plate was left away for 3 - 5 minutes, to allow surface moisture to dry before applying the discs. By using sterile forceps, under aseptic condition the antibiotic discs were applied into agar surface.

The discs were pressed down with a sterile needle or forceps to make contact with the surface of media. The plates were incubated inverted in the incubator at 37°C overnight. The plates were examined for inhibition zones, size measured in mm (APP-2) [19].

Gram negative strains were subjected to Modified Hodge test (MHT) for phenotypic detection of gene resistant. This test was performed as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2012 [20]. The suspension of ATCC *E. coli* 25922 and *Pseudomonas* 27853 and *Proteus* spp, *Acinetobacter* and *Klebsiella* spp were prepared in comparison to 0.5 Mc Farland standard in 5 mL of sterile saline using the direct colony suspension.

### Molecular detection of genes in multi drug resistance isolates (Gram negative)

Molecular techniques, primarily based on PCR, had been the reference standard for the identification and differentiation of resistance genes depended on the excellent specificity, sensitivity, accuracy and rapidity of these methods. If identification of gene was required for epidemiological purposes, then the PCR products.

These techniques generated results within 2 - 4 hours, or even less as for this study because we used conventional PCR technique.

DNA was extracted from bacterial colonies using the boiling lysis method. Strains were streaked onto Nutrient agar (Himedia, India) and grown overnight at 37°C. A loop full of bacterial growth was suspended in 1.5 ml of sterile deionized water in Eppendorf tube, incubated at room temperature for 5 minutes and then was pelleted by centrifuge at 13200 rpm for 2 minutes and the supernatant was removed. The cell pellet was suspended in 200 µl of deionized water, then the tubes were heated in water bath at 95°C for 10 minutes. The tubes were mixed by vortex for 10 seconds, then they were cooled to room temperature and centrifuged for 2 minutes at 13200 rpm. Following centrifugation, the supernatant was transferred to sterile Eppendorf tubes. The optical densities of the all samples were measured at 260 and 280 nm using Nano drop to determine DNA purity and quantity. DNA was stored at-20°C [21].

| Gene | Sequence (5' - '3) | Annealing Temp(°C) | Fragment (bp) |
|---|---|---|---|
| blaSHV | F: ATGCGTTATATTCGCCTGTG<br>R: AGATAAATCACCACAATGCGC | 56 | 896 |
| blaCTX-M-1 | F: CCGTTTCCGCTATTACAAACCGTTG<br>R: GGCCCATGGTTAAAAAATCACTGC | 56 | 944 |
| blaNDM | F: GCAGCTTGTCGGCCATGCGGGC<br>R: GGTCGCGAAGCTGAGCACCGCAT | 56 | 782 |

***Table 1:*** *Primer sets for amplification of resistance determining genes [22].*

### Statistical analysis

Data analysis was carried out using the Statistical Package of Social Sciences (SPSS) program version 25, Chi squire test was used and significance differences (P value) was adjusted with confidence interval (CI) 95% ($P < 0.05$ was considered significant).

Pearson's correlation was used to test the study hypothesis, and then the results of the analysis were displayed on tables and figures.

## Results

Fifty patients participated in our study, of those 37 (74%) were males and 13 (26%) were females, 36 patients (72%) were between the age of (60 - 80), all patients participating in this study had type 2 diabetes except for one patient who had type 1, they all use insulin to control diabetes and use antibiotics, 80% of them have diabetes for more than 10 years, and 86% had subcutaneous ulcers for more than a month, 80% of these ulcers range in size from 2 to 5 cm (Table 2). Forty two samples showed bacterial growth in culture out of 50 samples for diabetic foot ulcer patients 27 of them show Mono-microbial and 15 of them show Poly-microbial infection (2 - 3) growth (Figure 1) The most common isolated bacteria was *Proteus mirabilis* (80.0% of cases) which were aerobic Gram negative bacteria followed by *Enterococcus faecalis* (63.2% of cases) and *Staph aureus* (36.8% of cases) which are aerobic Gram positive bacteria (Table 3). The isolated bacteria were 43% aerobic Gram negative, 21% aerobic Gram positive, 19% anaerobic Gram positive and 17% anaerobic Gram negative (Figure 2). Bacterial isolates along with their antimicrobial susceptibility pattern shown in table 4 and 5. The distribution of resistance genes according to antibiotic groups were shown in table 6. Regarding the MDR isolates, all of the isolated gram negative bacteria showed resistance to three or more groups or antibiotics, (the commonest MDR Gram negatives were *Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Klebsiella pneumoniae, Acinetobacter baumannii, Escherichia coli).*

| Variables | N = 50 | Percentage |
|---|---|---|
| **Gender** | | |
| Male | 37 | 74 |
| Female | 13 | 26 |
| **Age** | | |
| 40-60 | 14 | 28 |
| 60-80 | 36 | 72 |
| **Type of diabetes** | | |
| Type 1 | 1 | 2 |
| Type 2 | 49 | 98 |
| **Duration of diabetes** | | |
| 5-10 Years | 10 | 20 |
| 10-15 Years | 20 | 40 |
| ≥15 Years | 20 | 40 |
| **Size of the wound** | | |
| < 2 cm | 4 | 8 |
| 2 - 5 cm | 40 | 80 |
| > 5 cm | 6 | 12 |
| **Depth of the wound** | | |
| Superficial | 7 | 14 |
| Subcutaneous | 43 | 86 |
| **Duration of ulcer** | | |
| ≤ 1 Month | 7 | 14 |
| > 1 Month | 43 | 86 |

***Table 2:*** *Patient's data.*

| | | N | Percent | Percent of Cases |
|---|---|---|---|---|
| Isolated Aerobic Gram-negative bacteria | *Bacteria* | 36 | 52.9% | 80.0% |
| | *Proteus Mirabilis* | 20 | 29.4% | 44.4% |
| | *Proteus Vulgaris* | 4 | 5.9% | 8.9% |
| | *Pseudomonas Aeruginosa* | 3 | 4.4% | 6.7% |
| | *E. coli* | 1 | 1.5% | 2.2% |
| | *Acinetobacter Baumannii* | 3 | 4.4% | 6.7% |
| | *Klebsiella Pneumoniae* | 1 | 1.5% | 2.2% |
| Total | | 68 | 100.0% | 151.1% |
| Isolated Aerobic Gram-positive bacteria | *Staph aureus* | 7 | 36.8% | 36.8% |
| | *Enterococcus faecalis* | 12 | 63.2% | 63.2% |
| Total | | 19 | 100.0% | 100.0% |
| Anaerobic Gram negative | *Bacteroides Fragilis* | 3 | 6.1% | 6.1% |
| Total | | 3 | 100.0% | 100.0% |
| Anaerobic Gram positive | *Clostridium* spp | 4 | 8.2% | 8.2% |
| Total | | 4 | 100.0% | 100.0% |

***Table 3:*** *The isolated bacteria.*

| | | N | Percent | Percent of Cases |
|---|---|---|---|---|
| Antibiotics resistance | Colistin | 39 | 11.3% | 93.9% |
| | Meropenem | 28 | 8.6% | 71.4% |
| | Gentamycin | 30 | 9.1% | 75.5% |
| | Cefepime | 41 | 11.8% | 98.0% |
| | Ceftriaxone | 41 | 11.8% | 98.0% |
| | Ampicillin | 41 | 11.8% | 98.0% |
| | Ciprofloxacin | 42 | 12.0% | 100.0% |
| | Ofloxacin | 41 | 11.8% | 98.0% |
| | Cefpodoxime | 41 | 11.8% | 98.0% |

***Table 4:*** *Percentages of isolated bacteria resistant to the antibiotics used in the study.*

| Drug | Bacteria | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | ***Staphylococcus aureus*** | | ***Proteus mirabilis*** | | ***Proteus vulgaris*** | | ***Pseudomonas aeruginosa*** | | *Escherichia coli* | | ***Actinobacteria*** | | ***Klebsiella pneumoniae*** | |
| | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** | **S** | **R** |
| Ceftriaxone | 1 | 6 | 1 | 19 | 3 | 1 | - | 2 | - | 1 | - | 3 | - | 1 |
| Cefpodoxime | 1 | 6 | 1 | 19 | 3 | 1 | - | 2 | - | 1 | - | 3 | - | 1 |
| Cefepime | 1 | 6 | 1 | 19 | - | 1 | - | 2 | - | 1 | - | 3 | - | 1 |
| Meropenem | 4 | 3 | 6 | 14 | 3 | 1 | - | 2 | - | 1 | - | 3 | - | 1 |
| Gentamycin | - | 7 | 9 | 11 | 3 | 1 | - | 2 | - | 1 | - | 3 | - | 1 |
| Ofloxacin | 1 | 6 | 1 | 19 | 3 | 1 | - | 2 | - | 1 | - | 3 | - | 1 |
| Colistin | 1 | 6 | 2 | 18 | 3 | 1 | - | 2 | - | 1 | - | 3 | - | 1 |

***Table 5****: Bacterial isolates along with their antimicrobial susceptibility pattern.*

*S: Sensitive, R: Resistant.*

| Genes | Fluoroquinolones group (11) | Polymyxin group (11) | Aminoglycoside group (8) | Cephalosporin group (7) | Carbapenem group (6) | Total (43) |
|---|---|---|---|---|---|---|
| bla CTX-M-1 | 5 (45.5%) | 5(45.5%) | 5 (62.5%) | 4 (57.1%) | 4 (66.6%) | 53.5% |
| bla SHV | 5 (45.5%) | 5 (45.5%) | 4 (50%) | 5 (71.4%) | 4 (66.6%) | 53.5% |
| bla NDM | 5 (45.5%) | 5 (45.5%) | 4 (50%) | 5 (71.4%) | 4 (66.6%) | 53.5% |

***Table 6:*** *The distribution of resistance genes according to antibiotic groups.*

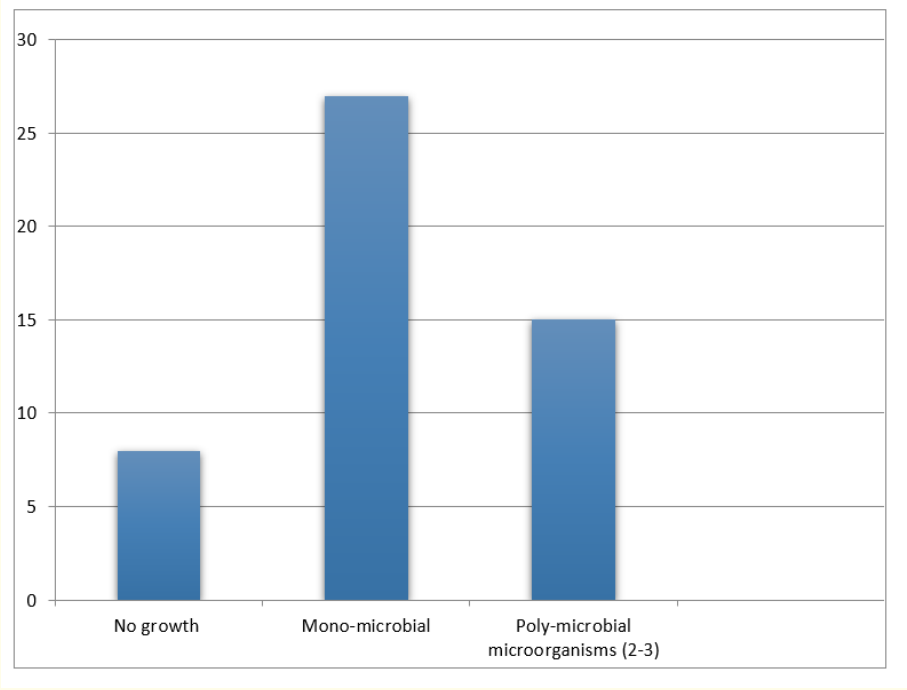


***Figure 1:*** *Culture results of 50 diabetic foot ulcers.*

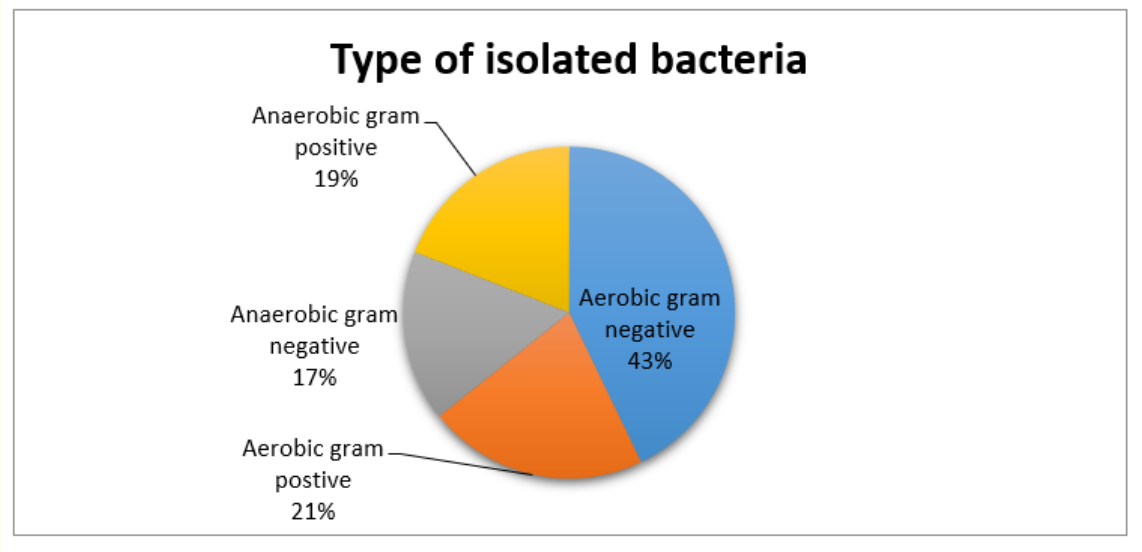


***Figure 2:*** *Type of isolated bacteria.*

By applying the PCR methods the commonest MDR isolated bacteria tested for the presence of B-lactamase genes, including ESBL genes (blaSHV and blaCTX-M-1), and carbapenemase-encoding blaNDM gene.

Extended spectrum beta lactamase (ESBL) bla SHV detected in 83% of isolated Gram-negative bacteria followed by extended-spectrum (ESBL) bla CTXM-1and Carbapenemase gene bla NDM; 66% and 61% respectively (Table 6 and figure 3-5).

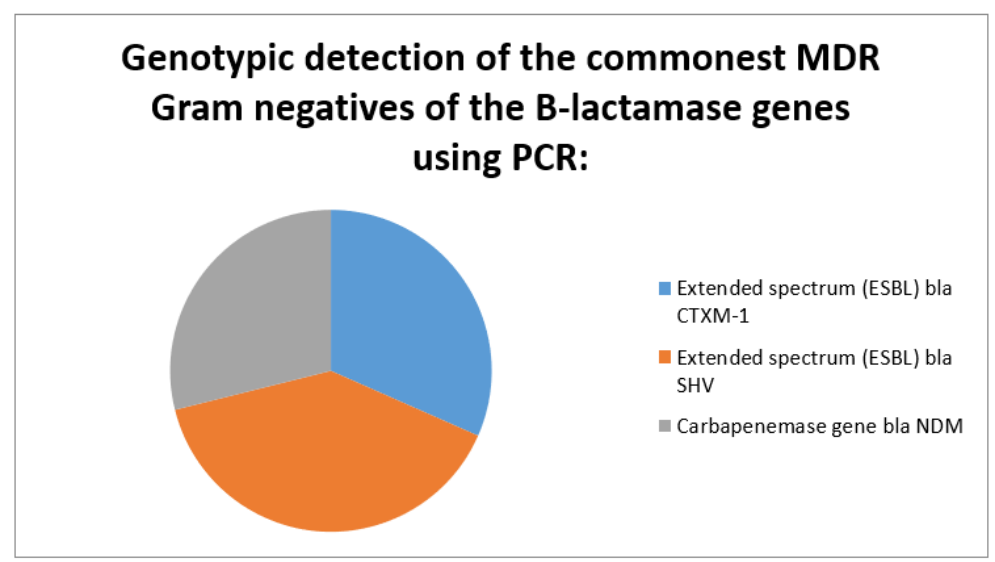


***Figure 3:*** *Genotypic detection of the commonest MDR gram negative of the B-lactamases genes using PCR.*

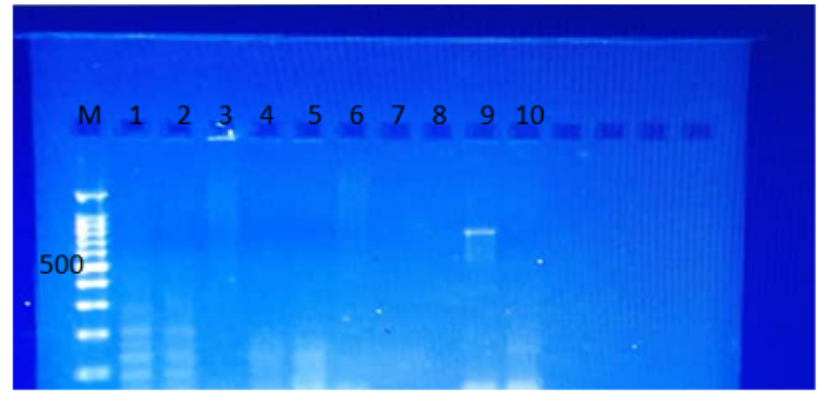


***Figure 4:*** *Gel photograph of amplification of DNA of the most common gram negative bacteria by PCR (bla SHV) gene results (896 bp): Lane M DNA ladder (100 bp) lane 1 -ve control, lane 9 shows positive result (amplified DNA of Proteus mirabilis), lane 2, 3, 4, 5, 6, 7, 8, and 10 show negative results.*

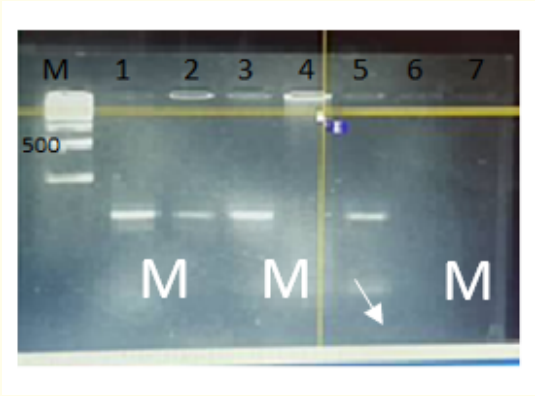


***Figure 5:*** *blaCTX-M-1, DNA results (944bp) on 1% agarose gel. Lane M shows 100 bp DNA marker, lane 1 shows negative control, lanes 4 show positive results , amplified DNA of (Pseudomonas aeruginosa), lane 2, 3, 5, 6, 7 shows negative results.*

## Discussion

According to the international diabetes federation (IDF) report of 2019, 19.4 million adults aged over 20 years and 25,800 aged less than 20 years have diabetes mellitus (DM) in Africa. Furthermore, the prevalence in Africa is expected to increase to 28 million by 2030 [23]. In a systematic review, the global prevalence of diabetic foot ulcer (DFU) was 6.3%, while in Africa it was found to be 7.2% [24]. A cohort study in Sudan reported a prevalence of 18.1% [25]. The higher prevalence of DFU makes it a major health problem in Sudan. Diabetic foot infections were associated with increased risk of amputations. Therefore, understanding the microbial prevalence and therapeutic approaches will help in reducing the duration of hospital stay and the need of amputation [26].

Among our patients, the majority (99%) had type two diabetes mellitus. Similar results were reported by Ismail., *et al*, Zubair., *et al*. and Gadepalli., *et al* [27-29]. This indicated that DFU was more common in patients with type 2 diabetes mellitus. Moreover, 82% of the patients had DM for more than 10 years with 41% had DM for 15 years or more. Choucair., *et al*. concluded that diabetic foot infections appear approximately after 17 - 20 years of the initial diagnosis [30]. The majority (86%) of our patients had the ulcer for more than a month. Sharma., *et al*. reported similar findings [31]. However, a study in north India reported different results with 56.6% patients had the infections for less than a month and 43.4% reported to have the infections for a month or more [27].

Diabetic foot infections are usually Poly-Microbial in nature [32]. Previous studies had showed high prevalence rates (83.8%, 83.7%) of poly-microbial infections [32,33]. In contrast, our study showed higher mono-microbial cultures than poly-microbial cultures (27 vs 15 cultures). Studies in Sudan, Egypt and India reported similar findings [27,34-37]. Patient's exposure to antibiotics around the time of sample collection can possibly explain the low poly-microbial infection rates in our study.

All the isolated pathogens in our study were bacteria. Alodaini., *et al*. reported fungal growth in Saudi patients with diabetic fool infections [38]. Aerobes were isolated more than anaerobes. Similar results were reported by Ako-Nai., *et al*. and Citron., *et al*. [33,39].

The majority of the ulcers in our study were deep (subcutaneous). We found significant association between the depth of the wound and the growth of organism in the culture (P value less than 0.001). Furthermore, significant association was found between the depth of the ulcer and the type of bacteria isolated (P value of 0.017). Ako-Nai., *et al*. [33] found that 54% of gram-positive organisms were cultured

from superficial swabs but no significant association was found between the depth of wound and the culture growth [39]. Gram-negative organisms were more frequently isolated from our patient's ulcers. Badawi., *et al*. in his study among Sudanese patients reported that gram-negative bacilli were the most predominant isolates [34]. Studies from India by Zubair., *et al*. and Shahi and Kumar., *et al*. reported similar findings [27,40]. While, Sharma., *et al*. reported that 73.6% of his isolates were aerobic gram-positive cocci [31].

We found that *Proteus mirabilis* was the most commonly isolated organism followed by *Enterococcus faecalis* and *Staph aureus*. Previous study in Sudan reported that commonest isolated organism [34]. Kamel., *et al*. and Gadepalli., *et al*. reported similar findings [29,36]. Other studies reported gram-negative organisms (*Escherichia coli* and *Pseudomonas aeruginosa*) were the most dominant isolates [30,41]. Studies from India reported *Staph Aureus was* the most predominant isolated organism [27,42,43]. *Clostridium* spp were the most commonly isolated anaerobes in our study. A study by Ismail., *et al*. reported *Ruminococcus* spp as the most abundant anaerobic isolates followed by *Bacteroides* spp [28].

Most of the isolated cultures were resistant to multiple antibiotics. All of the isolated organisms were resistant to ciprofloxacin. Gaurav Dalela in their work reported ciprofloxacin resistance of 54.8% [44]. Organisms showed the least resistance to meropenem (71.4%) and gentamycin. Previous studies showed gentamicin resistance of 50% and 55.5% [49,56]. The high resistance percentages indicate inappropriate prescription of antibiotics by physicians and the misuse of antibiotics by the patients without medical consultation.

Going further, our *Staph aureus* showed high rates of resistance to most antimicrobial with meropenem being the most sensitive drug to *Staph aureus*. *Proteus* spp were more sensitive to gentamicin than other antimicrobial drugs. Most of our *Pseudomonas aeruginosa* isolates were resistance to most of the tested antimicrobial including meropenem. Jain and his colleagues reported that most of *Staphylococcus* isolates showed highest sensitivity to gentamycin while 70% of *Pseudomonas* were sensitive to meropenem [37]. Sharma., *et al*. reported gentamycin as the most sensitive drug to gram positive cocci [31]. While Mohamed Taher and his team noticed that *Proteus* spp and *Pseudomonas aeruginosa* were highly resistant to amikacin while *Klebsiella pneumoniae* were mostly resistant to cefoxitin and levofloxacin [35]. Kamel., *et al*. reported that all his *Staph aureus* were resistant to ampicillin and 96.1% were sensitive to vancomycin [36]. In addition, Ali., *et al*. concluded that *Escherichia coli* and *Pseudomonas aeruginosa* had the highest level of multiple antibiotic resistance in his study [63]. In our study, we showed high resistance of Gram-negative isolates to Ceftriaxone. Previous study showed similar findings [45].

In our study, direct relationship was found between the duration of ulcer and multidrug resistance bacteria. A study in North India reported that 78.5% of patients with ulcer for more than a month had MDR infection [49]. While Ismail., *et al*. reported no association between the presence of MDR and the severity of diabetic foot infection [28].

Regarding MDR Gram-negatives, using PCR *blaSHV* gene was the most predominant genotype. Badawi., *et al*. in his study reported *blaCTX-M* as the most dominant gene with 100% of ESBL producers being *Klebsiella* or *Proteus* spp [34]. Furthermore, Kamel., *et al*. reported detection of *blaCTX*-gene is all of his ESBL producers, and *blaCTX-M* in 37.5%. In addition. Kamel reported that highest ESBL production was found in *Klebsiella* spp [36]. While Gadepalli and his colleagues reported that 54% of *Escherichia coli* were ESBL producers [29]. Another study reported *Escherichia coli* as the most common ESBL producer followed by *Proteus vulgaris* [44].

## Limitations of the Study

The study had some limitations in the data with the process of data collection lasted only for 6 months with 50 diabetic septic foot patients being recruited into the study and this was because the study was time-bound. Furthermore. In addition, the method used in collecting the specimens was only deep pus swabs. Patients who were enrolled in this study might have been exposed to prior antibiotic treatment at primary/peripheral centers.

## Conclusion

Our study showed higher incidence of mono-microbial DFI. We noticed the predominance of aerobic gram- negative in patients with diabetic septic foot infections. *Proteus* spp were the most commonly isolated organisms. High rates of resistance toward most of the tested antibiotics were reported. Meropenem and gentamycin were the most effective against these bacteria. We found significant association between the duration of ulcer and the presence of multidrug resistance implying that longer duration of ulcer can be a possible risk factor for developing of multidrug resistance. Furthermore, direct relationship was found between the depth of wound and growth of culture.

Gram-negative organisms expressed high rates of extended spectrum B-lactamase genes. *BlaSHV* was the most detected gene followed by *blaCTX-M* and carbapenems gene *blaNDM.*

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**Volume 18 Issue 9 September 2022**