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# Prevalence of the beta-lactamase producing bacteria in diabetic foot infection of the Egyptian diabetic patients

Abdelgyed lela[1], Ahmed Radwan[2], Mohamed Fouda[3], sehamShash[4], Mahmoud Hazaa[5].

[1]SpecialistMicrobiology at Elresala Lab.
[2]Genetics and Cytology department, National research Center(NRC), Giza, Egypt.
[3]Assistant professor of Microbiology and immunology at liver and digestive surgery center at the Faculty of Medicine Mansoura University.
[4]Professor of microbiology at faculty of science Banha University.
[5]Professor of microbiology at faculty of science banha university.

## ABSTRACT

Adiabatic foot infection is one of the most feared complications of Diabetes mellitus. Many studies have reported on the bacteriology of Diabetic Foot Infections (DFIs) over the past 25 years, but the results have been varied and often contradictory. Determination the prevalence of beta-lactamase producing bacteria in diabetic foot infections and study the pattern of antibiotic resistance to these isolates, which were received from Mansoura university Hospital by means of antimicrobial susceptibility tests and phenotypic methods. Then confirmed by using Polymerase chain reaction (PCR) to detect Extended-spectrum beta-lactamases (ESBL) genes encodingresistance to beta-lactam antibiotics.

A total of 59 bacterial isolates were obtained from 56 patients with diabetic foot ulcers. The age group of these patients ranged from 40 to 70 years. Gram-positive cocci were more prevalent (50.8%) than gram-negative bacilli (49.15%). The commonest isolate was *Staphylococcus.aureus* (33.9%). followed by 15.25 % *Pseudomonas.aeruginosa,* 11.86 %*Proteus.mirabilis*. The antibiotic sensitivity profiles of theisolated bacteria ,showed that most isolates were resistant to different beta lactams antibiotics .The application of Polymerase chain reaction (PCR) showed that Extended-spectrum beta-lactamases ESBL) producers and the blaTEM, blaSHV, blaCTX-M and blaOXA genes were detected.

## 1.  Introduction

Diabetes is a metabolic disorder of the endocrine system which plagues approximately 17 million people nationwide Each year over 700,000 new cases are diagnosed; 12,000 to 14,000 of which are children, teenagers and young adults. Diabetes is often accompanied by serious complications, and is predicted to affect 239 million people worldwide by 2010 [1].

Fifteen percent of all diabetics develop a foot ulcer at some point in their lives which is highly susceptible to infections and that spreads rapidly, leading to overwhelming tissue destruction and subsequent amputation [2].

Diabetic foot infections (DFIs) are associated with significant mortality and morbidity and are the leading cause of non-traumatic lower extremity amputations [3]. Patients with diabetes are particularly susceptible to foot infection primarily because of neuropathy, vascular insufficiency, and diminished neutrophil function [3].

Mostly the diabetic foot infections are mixed bacterial infections are generally caused by aerobic Gram-positive like S. aureus,Staphylococcus epidermides and Streptococcus  pyogens and by Gram- Negative Enterococci like E. coli, Klebsiella species and Proteus [4]. The proper management of these infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility testing. Sometimes, initial management comprises empirical antimicrobial treatment based on susceptibility data [5].

Many of these microorganisms that cause infected DFU are developing resistance to commonly used antibiotics largely due to their indiscriminate use, Infection with multidrug resistant organisms (MDRO) may increase the duration of hospital stay, cost of management and may cause additional morbidity and mortality [6].

In recent years, there has been an increase in the incidence and prevalence of Bacteria that produce enzymes called extended-spectrum beta-lactamases (ESBLs) [6].

Beta-lactamases are enzymes produced by some bacteria when antibiotics are present in the environment that provide resistance to -lactam antibiotics like penicillins, cephamycins, and carbapenems, Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure. These antibiotics all have a common element in their molecular structure: a four-atom ring known as a -lactam. Through hydrolysis, the lactamase enzyme breaks the -lactam ring open, deactivating the molecule's antibacterial properties [7].

The gene encoding the -lactamase showed a mutation of a single nucleotide compared with the gene encoding SHV-1. Other closely related TEM-1 and TEM-2 -lactamase are soon discovered [8]. These new -lactamase are termed as extended spectrum -lactamases (ESBLs) and detailed in the authorative website of the nomenclature of ESBLs by George Jacoby and Karen Bush (http://www.lahey.org/studies/ webt.htm).

found that ESBLs have the ability to inactivate -lactam antibiotics containing an oxyamino-group such as oxyamino-cephalosporins (eg, ceftazidime, ceftriaxone, cefatoxime) as well as the oxyamino-monobactam (eg. aztreonam) but they are not active against cephamycins and carbapenems [9]. Generally, they are inhibited by -lactamas-inhibitors such as clavulanate and tazobactam.

ESBLs have been found in a wide range of Gram-negative rods, the vast majority of strains expressing these enzymes belong to the Enterobacteriaceae family, especially *Klebsiella* spp., *E. coli* and other genera such as *Citrobacter*, *Enterobacter*, *Morganella*, *Proteus*, *Providencia*, *Salmonella*, *Serratia*, and *Pseudomonas* [10].

More than 150 types of ESBLs have been described and the majority of these enzymes belong to the TEM and SHV families [11].

Stated that the majority of plasmid-mediated beta-lactamases (TEM-1 or less frequently TEM-2) are broad-spectrum -lactamases which do not hydrolyze oxyamino-cephalosporins or aztreonam. The Cefatoxime (CTX-M) family was described in 1992 and considered to be the most dominant non-TEM or SHV. It is recognized as a rapidly growing family of ESBLs hydrolyzing cefotaxime more than ceftazidime [12].

The genes that encode ESBLs are frequently placed on the plasmids which comprise genes of resistance to aminoglycosides and sulfonamides [13].

The present study aimed to investigate the presence of beta-lactamase producing bacteria in diabetic foot infections, using Polymerase chain reaction (PCR) to detect genes encoding resistance to beta-lactam antibiotics.

## 2. MATERIAL AND METHODS

### Subjects
Thirty male and 26 female diabetic patients of different age attending the outpatient clinic of diabetic foot unit Mansoura University Hospitals (Dakhlia, Egypt) from January, 2014 to December 2014 and clinically diagnosed as having diabetic foot infections were included.

### Sample collection and processing
Swabs were collected from patient's wounds. The specimens were collected in a sterile container and transported to the microbiology diagnostics and infection control unit (MDICU), microbiology department. The samples were inoculated on blood agar plates. The plates were examined after 24 hours incubation at 37 when no growth appeared. The culture plates were reincubated for another 24 hours, plates with no growth was considered free from aerobic organism.

### Isolation and identification of bacteria
Bacterial isolates were identified according to the colonial characteristic appearance, hemolytic patterns, microscopically by Gram's stain and biochemically according to Berge's Manual of Determinative Bacteriology [14]. Seven different characterized bacterial strains including *Escherichia coli, Klebsiella pneumonia, Proteus mirabilis, Proteus vulgaris, Pseudomonas aeruginosa, Staphylococcus aureus,*and *Staphylococcus epidermidis* were obtained .The strains were maintained on Nutrient agar salants. All the bacterial isolates were tested against 16 different antibiotic discs by the standard disc diffusion [15]. The zone diameter (ml.) of the sensitivity was interpreted based on the CLSI guidelines [16].The following antibiotic discs were used, P: penicillin (G), AMC: amoxicillin /clavulanic acid, SAM: Ampicillin/sulbactam, CEP: cefoperazone, CXM: cefuroxime, CAZ: ceftazidime, CTX: cefotaxime. Isolates found to be resistant to any of their beta-lactams antibiotics groups were selected for Extended-spectrum beta-lactamases (ESBLs) detection [17].

## 3. Results and discussion

Out of the 56 diabetic foot patient, 48 had positive bacterial cultures (37 patient had mono microbial infection and 11 had poly microbial infection., A total of 59 multi drug resistant isolates were obtained as shown in table **(1).**

Table (1): The distribution number of pathogenic bacteria isolates from diabetic foot ulcer samples

| Pathogenic bacterial isolates | No. of bacterial isolates | Percentage of distribution |
|---|---|---|
| S.aureus | 20 | 33.9 % |
| S.epidermidis | 10 | 16.95 % |
| K.pneumoniae | 6 | 10.17 % |
| E-coli | 4 | 6.78 % |
| P.aeruginosa | 9 | 15.25 % |
| P.vulgaris | 3 | 5.09 % |
| P.mirabilis | 7 | 11.86 % |
| Total | 59 | 100 % |

**3-1-Phenotypic methods for Extended-spectrum beta-lactamases (ESBLs) detection (including Two methods)**

**3-2- The antibiotic activity of the bacterial isolates: (<u>Methods 1</u>)**

**Table (2):** The sensitivty of antibiotics (mm) on the multiresistant *K.pneumoniae* isolates.N(6)

| Antibiotic / Isolate No. | Inhibition zone diameter (mm) | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | P (10ug) | AMC (30ug) | SAM (20ug) | CEP (75ug) | CXM (30ug) | CAZ (30ug) | CTX (30ug) | CRO (30ug) | FEP (30ug) | CFR (30ug) | MEM (10ug) | PRL (100ug) | AK (30ug) | LEV (5ug) | DA (2ug) |
| *Control | S 29 | S 17 | S 15 | S 21 | S 18 | S 18 | S 23 | S 21 | S 18 | S 18 | S 17 | S 18 | S 17 | S 17 | S 21 |
| 1 | R | 12(M) | R | 11(M) | R | 21(S) | R | R | 25(S) | 10(M) | 20(S) | R | 21(S) | 15(M) | R |
| 2 | R | 11(M) | R | 13(M) | R | 20(S) | R | R | 23(S) | 11(M) | 19(S) | R | 22(S) | 12(M) | R |
| 3 | R | 10(M) | R | 10(M) | R | 19(S) | R | R | 20(S) | 12(M) | 20(S) | R | 20(S) | 14(M) | R |
| 4 | R | 15(M) | R | 12(M) | R | 19(S) | R | R | 21(S) | 14(M) | 19(S) | R | 25(S) | 10(M) | R |
| 5 | R | 14(M) | R | 14(M) | R | 20(S) | R | R | 25(S) | 15(M) | 23(S) | R | 22(S) | 13(M) | R |
| 6 | R | 13(M) | R | 15(M) | R | 25(S) | R | R | 24(S) | 13(M) | 22(S) | R | 20(S) | 12(M) | R |

P: penicillin(G), AMC: amoxicillin / clavulanic acid,SAM:Ampicillin/sulbactam,CEP:cefoperazone,CXM:cefuroxime,CAZ:ceftazidime,CTX:cefotaxime,CRO:ceftriaxone,FEP:Cefepime, CFR:Cefadroxil,MEM: Meropenam, PRL:piperacillin,AK: amikacin,LEV:Levofloxacin,DA:Clindamycin, S(sensitivity),R(resistant),M(moderate)
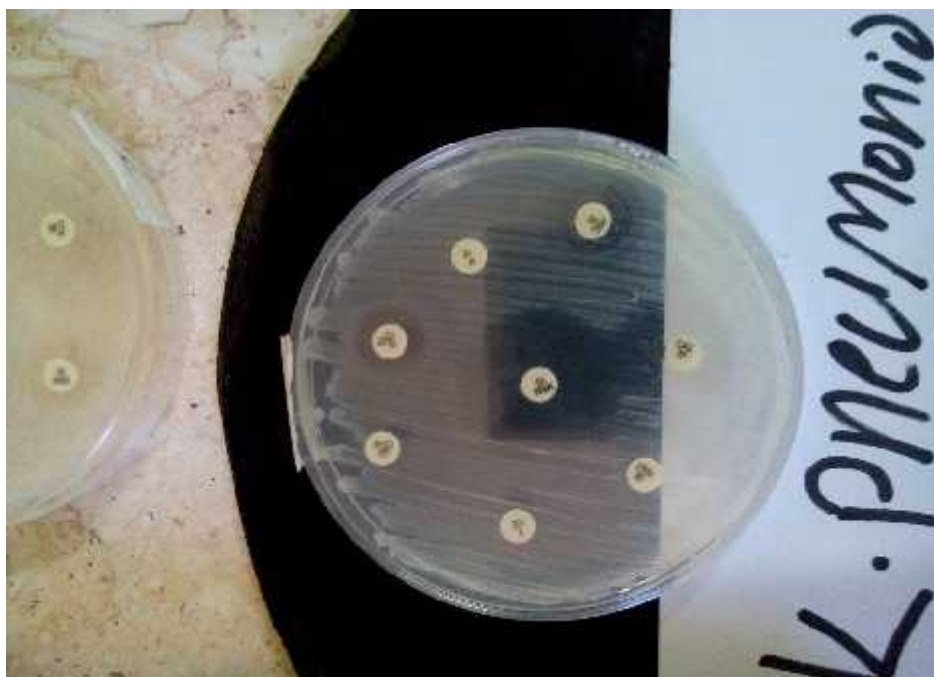


Fig (1): The resistant activity of *K. pneumoniae* on different antibiotic discs (CXM, CRO, DA, P,PRL,CTX,SAM) using Bauer-Kirby method. AMC, CFR, CEP, LEV are moderate antibiotic.MEM , AK, CAZ,FEP are sensitive antibiotics.

Fig (2): The resistant activity of *K. pneumoniae*on different antibiotic discs (CXM, CRO, DA, P,PRL,CTX,SAM) using Bauer-Kirby method. AMC, CFR, CEP, LEV are moderate antibiotic.MEM , AK, CAZ,FEP are sensitive antibiotics.

Table (3): The antibiotic activity (%) on the selected multiresistant isolates

| Antibiotic / Bacteria | P | AMC | SAM | CEP | CXM | CAZ | CTX | CRO | FEP | CFR | MEM | PRL | AK | LEV | DA |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| *E.coli* (n=4) | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 100 | 100 | 100 |
| *K. pneumonia* (n=6) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 100 | 0.0 | 100 | 0.0 | 100 | 0.0 | 0.0 |
| *P.aeruginosa* (n=11) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 100 | 100 | 100 | 0.0 | 100 | 0.0 | 0.0 |
| *Pr.mirabilis* (n=7) | 0.0 | 100 | 0.0 | 100 | 100 | 100 | 0.0 | 0.0 | 100 | 0.0 | 100 | 0.0 | 100 | 0.0 | 0.0 |
| *Pr.Vulgariss*(n=4) | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 100 | 100 | 100 |
| *S.aureus*(n=20) | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 |
| *S.epidermadis* (n=10) | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 100 | 0.0 | 0.0 | 0.0 | 0.0 | 100 | 0.0 | 100 | 100 | 0.0 |

P: penicillin(G), AMC: amoxicillin / clavulanic acid,SAM:Ampicillin/sulbactam,CEP:cefoperazone,CXM:cefuroxime,CAZ:ceftazidime,CTX:cefotaxime,CRO:ceftriaxone, FEP: Cefepime, CFR:Cefadroxil, MEM: Meropenam, PRL:piperacillin, AK: amikacin, LEV:Levofloxacin,DA:Clindamycin

### 3-3-Detection of beta lactamase activity (Rapid acidimetric filter paper test):(Methods 2)

The test was performedto single bacterial isolates of *E.coli, K. pneumonia*, *P. aeruginosa, Pr. Mirabilis, Pr. Vulgaris*, *S. epidermadse*, *S. aureus*showingresistance to beta-lactam antibiotics. All isolates gave positive results (yellow color) indicating suspicion for ESBL production. Thus, the genotypic methods were applied to determine the genes responsible for production ESBLs in a single isolate.

### 3-4-Genotypic detection of beta- lactamase by using PCR amplification(Methods 3)

The seven selected ESBL producing isolates (n=7) in vitro were cultured (figure 3) , the DNA were extracted(figure 4) and amplified by polymerase chain reaction (PCR) to detect ESBL genes using bla-SHV, bla-TEM and bla-CTX-M, bla-OXA primers. The PCR products were analyzed by field gel electrophoresis and visualized (figures 5, 6, 7) and the result that obtain from this amplification were recorded in (table 4)

Fig (3): Culturing the  -lactam resistant strains on nutrient broth medium (20 mL) for the genomic DNA extraction. Code: C, control (Nutrient broth medium without inoculation); 1, *Escherichia coli* ; 2, *Staphylococcus aureus*; 3, *Staphylococcus epidermidis*; 4, *Klebsiellapneumoniae*; 5, *Pseudomonas aeruginosa* ; 6, *Proteus vulgaris*; 7, *Proteus mirabilis*.
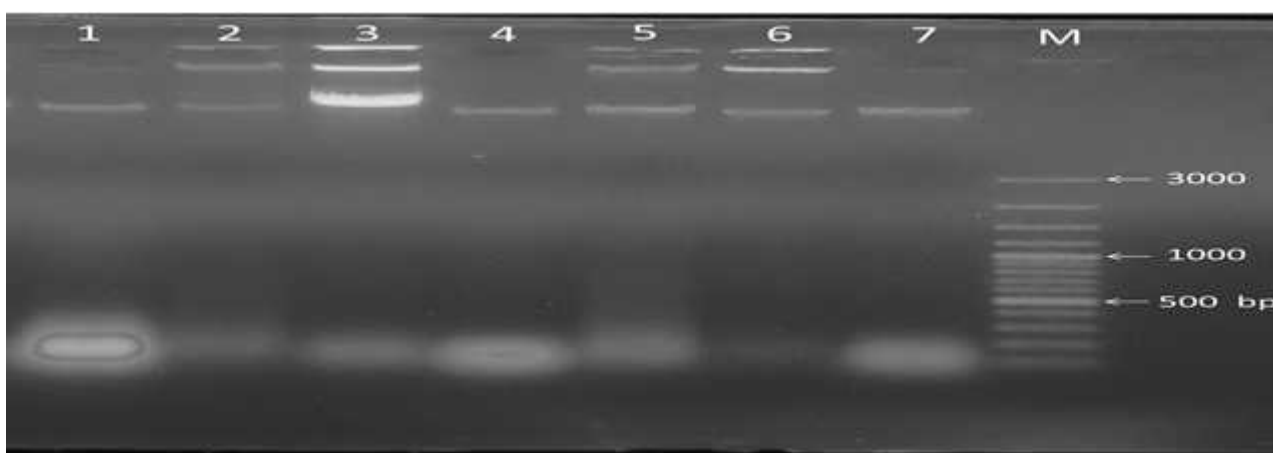


Fig (4): Agarose gel electrophoresisfor extracted DNA.Lane: 1, *Escherichia coli*; 2, *Staphylococcus aureus*; 3, *Staphylococcus epidermidis*; 4, *Klebsiellapneumoniae*; 5, *Pseudomonas aeruginosa*; 6, *Proteus vulgaris*; 7, *Proteus mirabilis*; M, DNA size marker (GeneRuler™ 100 bp Plus DNA Ladder, Thermo Scientific #SM0323).
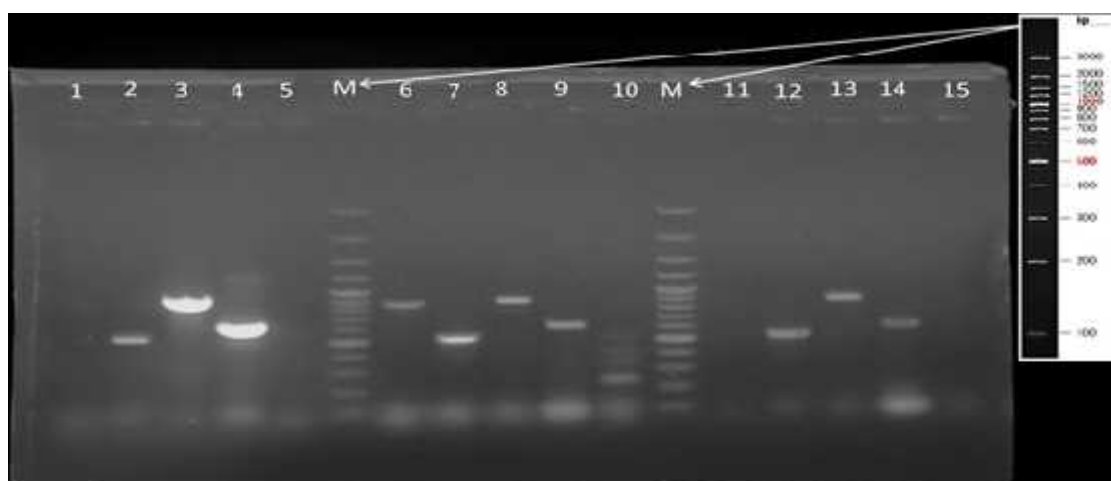


Fig (5):  PCR-based detection of  -lactamase genes at the *Escherichia coli* (Lane 1:5), *Staphylococcus aureus*(Lane 6:10) and *Staphylococcus epidermidis*(Lane 11:15). Lane 1, 6 and 11, specific primer of bla_TEM gene used to amplify PCR product  861 bp; Lane 2, 7 and 12, specific primer of bla_TEM gene used to amplify PCR product  526 bp; Lane 3, 8 and 13, specific primer of bla_SHV gene used to amplify PCR product  885 bp; Lane 4, 9 and 14, specific primer of bla_CTX-M gene used to amplify PCR product  600 bp; Lane 5, 10 and 15, specific primer of bla_OXA gene used to amplify PCR product  828 bp. Lane M, DNA size marker (GeneRuler™ 100 bp Plus DNA Ladder, Thermo Scientific #SM0323).
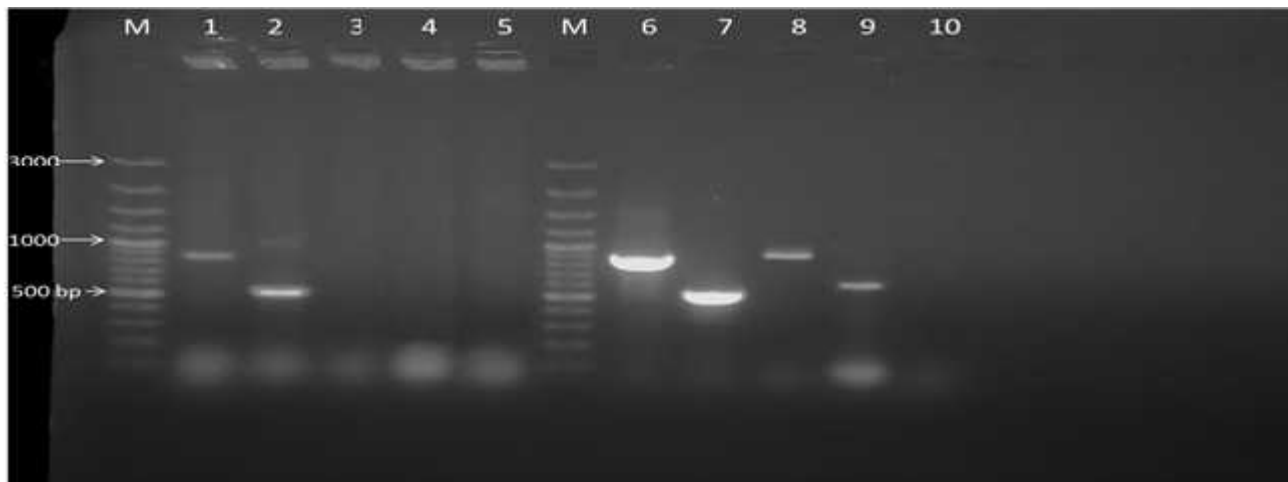
Fig (6): PCR-based detection of -lactamase genes at the *Klebsiellapneumonia*(Lane 1:5) and *Pseudomonas aeruginosa*(Lane 6:10). Lane 1 and 6, specific primer of bla_TEM gene used to amplify PCR product 861 bp; Lane 2 and 7, specific primer of bla_TEM gene used to amplify PCR product 526 bp; Lane 3 and 8, specific primer of bla_SHV gene used to amplify PCR product 885 bp; Lane 4 and 9, specific primer of bla_CTX-M gene used to amplify PCR product 600 bp; Lane 5 and 10, specific primer of bla_OXA gene used to amplify PCR product 828 bp. Lane M, DNA size marker (GeneRuler[TM] 100 bp Plus DNA Ladder, Thermo Scientific #SM0323).
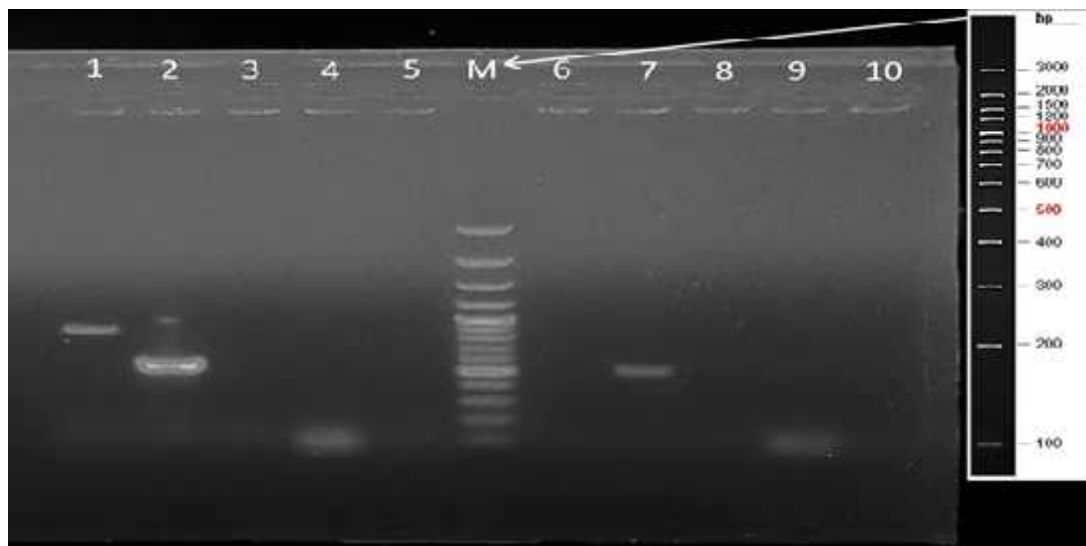


Fig (7): PCR-based detection of -lactamase genes at the *Proteus vulgaris* (Lane 1:5) and *Proteus mirabilis* (Lane 6:10). Lane 1 and 6, specific primer of bla_TEM gene used to amplify PCR product 861 bp; Lane 2 and 7, specific primer of bla_TEM gene used to amplify PCR product 526 bp; Lane 3 and 8, specific primer of bla_SHV gene used to amplify PCR product 885 bp; Lane 4 and 9, specific primer of bla_CTX-M gene used to amplify PCR product 600 bp; Lane 5 and 10, specific primer of bla_OXA gene used to amplify PCR product 828 bp. Lane M, DNA size marker (GeneRuler[TM] 100 bp Plus DNA Ladder, Thermo Scientific #SM0323).

Table (4): Results of the PCR of determination of the -lactamase genes.

| No. | Isolate Name | -lactam resistance genes | | | |
|---|---|---|---|---|---|
| | | bla_TEM | bla_SHV | bla_CTX-M | bla_OXA |
| 1 | *Escherichia coli* | + | + | + | - |
| 2 | *Staphylococcus aureus* | + | + | + | - |
| 3 | *Staphylococcus epidermidis* | + | + | + | - |
| 4 | *Klebsiellapneumoniae* | + | - | - | - |
| 5 | *Pseudomonas aeruginosa* | + | + | + | - |
| 6 | *Proteus vulgaris* | + | - | - | - |
| 7 | *Proteus mirabilis* | + | - | - | - |

As shown in (table 4) thebla_TEM gene were detected in all selected isolate (*E. coli, S. aureus, S. epidermidis, K.pneumoniae, P. aeruginosa , P. vulgaris* and *P. mirabilis*). Moreover, the bla_SHV and bla_CTX-M genes were also detected in four isolates (*Escherichia coli, Staphylococcus aureus, Staphylococcus epidermidis* and *Pseudomonas aeruginosa*) out of the seven selected isolates. However the bla_OXA were not detected in any of the tested isolates.

## Discussion

Diabetes is a group of metabolic diseases in which there are high blood sugar levels over a prolonged period.Untreated, diabetes can cause many complications [18]. Diabetes, with its increasing prevalence ,is regarded as a global health problem. Today, it affects approximately 171 million people worldwide and this number is estimated to reach 366 million in 2030 [19].

Diabetic foot infections (DFIs) are associated with significant mortality and morbidity and are the leading cause of non-traumatic lower extremity amputations [20]. Patients with diabetes are particularly susceptible to foot infection primarily because of neuropathy, vascular insufficiency, and diminished neutrophil function [2].

Mostly the diabetic foot infections are mixed bacterial infections are generally caused by aerobic Gram-positive like S. aureus, and Streptococcus pyogenes and by Gram- Negative Enterococci like E. coli, Klebsiella species and*Proteus* [4].

The proper management of these infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility testing. Sometimes, initial management comprises empirical antimicrobial treatment based on susceptibility data [5].

In recent years, there has been an increase in the incidence and prevalence of Bacteria that produce enzymes called extended-spectrum beta-lactamases (ESBLs) [6].

Beta-lactamases are enzymes produced by some bacteria when antibiotics are present in the environment that provide resistance to -lactam antibiotics like penicillins, cephamycins, and carbapenems, Beta-lactamase provides antibiotic resistance by breaking the antibiotics' structure.These antibiotics all have a common element in their molecular structure: a four-atom ring known as a -lactam. Through hydrolysis, the lactamase enzyme breaks the -lactam ring open, deactivating the molecule's antibacterial properties [7].

In this study bacteria were isolated from56 samples ofdiabetic foot ulcers from, 30 male patients (45 to 70 years) and 26 females(40 to 65 years).

This study has shown that maleswith DFU were predominant (30 patients,53.5 %) compared with women (26 patients,46.4%) which agreed with [21] and [22],Who reported the prevalence of DFIS between males than females .

However, our results were in disagreement with those of [23] who reported the prevalence of DFI between , females (54%) were female and 19 (46%) were males.

Our results reported 29 isolates of gram negative bacilli,and 30 isolates gram positive cocci. The gram negative isolates includedfour isolates of *Escherichia coli*, six isolates *Klebsiellapneumoniae*, two isolates *Proteus vulgaris*, eight isolates *Proteus mirabilis*, and nine isolates *Pseudomonas aeruginos*. The Gram-positive isolates were ten isolates *Staphylococcus epidermidis*, twenty isolates of *Staphylococcus aureus.*

This study has shown that the most common isolated Gram negative bacteriawere,5.09 % of *Proteus vulgaris, 15.25 % Pseudomonas aeruginosa, 6.78 % of E-coli, 10.17 %of Klebsiellapneomoniae , 11.86 % Proteus. mirabilis* followed by gram positive bacteria similar results were also reported by [24],who reported Gram negative bacteria to be the most frequently isolated pathogens(28.7%) followed by 13.8% gram positive bacteria.

Our study has shown that the 56 diabetic foot ulcers patients ( DFU) recovered 59 of aerobic bacteria of which Gram negative bacilli made up 29(49.15%) were recovered from 56 diabetic foot ulcers patientsand only Gram positive cocci made up 30(50.8%) of infections.

The most frequently isolated Gram negative bacteriawere, 5.09 % of *Proteus vulgaris, 15.25 % Pseudomonas aeruginosa,* 6.78 % of *E-coli,_10.17 %of Klebsiellapneomoniae ,* 11.86 % *Proteus mirabilis.* Also, show in this study *Pseudomonas aeruginosa* was the predominant Gram negative followed by *Proteus.mirabilis, Klebsiellapneomoniae, E-coli .*The most common gram positive cocciwas made up 30(50.8%) of infections, and especially *Staphylococcus aureus* 20(33.9%). followed by *Staphylococcus epidermidis* 10(16.95%).**This result** agrees with [25], who cultured a total of 454 specimens and isolated 1,607organisms, 427 Culture-positive specimens, 16.2% had growth of a single organism, while the rest were polymicrobial, with43.7% yielding four or more organisms.The predominant aerobic species was *S. aureus*, 76.6 % followed by S. *epidermidis*, accounted for 49.7%. Staphylococcus lugdunensis was cultured from 22 specimens Staphylococcus haemolyticus was recovered from 22 specimens then Staphylococcus simulans, Staphylococcus hominis, Staphylococcus hominis . Also, they reported Streptococci as the next most frequently cultured group and comprised 15.5% (177 of 1,145) of all aerobic strains with Streptococcusagalactiae accounting (48.6%) and the Streptococcus mitis and Streptococcus milleri groups accounting 33.8% . Gram-negative rods comprised 19.7% (225 of 1,145) of the aerobic organisms. Pseudomonas aeruginosa was the predominant species. Proteus mirabilis and Klebsiellaspecies were the next most often recovered gram-negative aerobes. Also, these results are in agreement with that of [23].

In our study the most common pathogens isolated were Gram-positive cocci, such as *Staphylococcus aureus*and *Staphylococcus saprophyticus* (CONS) and Gram-negative rods, such as *Proteus spp*. and Enterobacter spp. Although the findings of our study are consistent with the results of previous studies showing that Gram-positive bacteria were predominant in diabetic foot infections [26]. Other studies have reported that Gram-negative bacteria were predominant in particular regions [27]. The previous results were in disagreement with those of [22],who found that 107 DFU patients, recovered 312 aerobic bacteria, of which 191( 61.3%) were Gram-negative and only 121(38.7%) were Gram-positive isolates.

In present study meropenam and amikacin were the effective antibiotics against all Gram negative bacteria, and Levofloxacin were the effective antibiotics against all Gram positive bacteria. all the selected isolates were resistant to the tested antibiotics penicillin, also all Gram negative bacteria moderate sensitive to AMC antibiotics except *E.coli* resistant to AMC.

Our results comes in agreement with that of [28], as the percentage of sensitive Gram negative bacteria organisms to meropenam is 98.0%, amikacin 85.0%,95% Levofloxacin were the effective antibiotics against all Gram positive bacteria isolated and penicillin-G 0.0% resistant selected isolates.Also,the results of [29], go in line with our results, where all isolates were poorly susceptible to penicillin, but exhibited good susceptibility to meropenam.

Furthermore most of our isolates were resistant to the majority of tested beta lactam antibiotics, It is important to consider that some Gram-negative bacteria from the Enterobacteriaceae family have the ability to produce highly effective ß-lactamase enzymes, making them resistant to all ß-lactam antibiotics, In the present study, the rapid acidometric filter paper test to detect beta-lactamase activity, showed that all isolates give positive results (yellow colour) which is in agreement with the results of [30].

Molecular analysis in the present work showed that the blaTEM is the most predominant ESBL gene detected in seven tested isolates by using polymerase chain reaction (PCR).,the most predominant ESBL producers in this study are *E. coli.*, the blaTEM was the commonest genotype followed by blaCTX-M and blaSHV and do not detected the genotype blaOXA in the tested isolates., the predictive factors that were associated with blagene (CTX-M , TEM , SHV )positivity in cefotaxime resistant *E. coli, S. epidermadse, S. auerus, P. aeruginosa* members isolated from D.F.U patients. This finding is in agreement with [21], who examined the ESBL production clinical isolates from D.F.U were found the bla gene positivity was 89.3 %, of which bla CTX-M positivity was higher(81.8%),followed by blaTEM (50.0%) and blaSHV (46.9%) we also found higher blaCTX-M, blaTEM, and blaSHV positivity in bacteria isolated from DFU patients. the most prevalent ESBL gene was blaCTX-M(81.8%),followed by 50% blaTEM and46.9% blaSHV,in the cefotaxim – resistant Enterobacteriaceae members *(E. coli and Klebsiellasp)* isolated from DFU patients. The genotypic methods helped us to confirm the genes responsible for the production of ESBLs inasingel isolate. to the best of our knowledge, no such type of study has been reported from isolates of DFUs, however, blaCTX-M as the most prevalent and widely disseminated gene in the DFU bacterial population has been reported in our previous findings.

## 4. Conclusions

The conclusions of this study which appeared can be summarized as fellow:

1- In conclusions the present study confirmed the high prevalence of multidrug-resistant pathogens in diabetic foot ulcers. ESBL constitutes a major threat to currently available beta-lactam therapy, leading to complications in DFUs.

2- Aminoglycosides, cephalosporin, and b-lactam inhibitor drugs would probably be more appropriate empirical agents after establishing the patient's history of previous antibiotic use.

3-The findings from the present study revealed high prevalence of ESBL in diabetic foot unit,Mansoura University specialized medical hospital, (Dakahlia, Egypt)*Staphylococcus aureus*has the highest prevalence followed by *Staphylococcus epidermidis*, *Escherichia coli, Klebsiellapneumoniae, Pseudomonas aeruginosa, Proteus vulgaris, Proteus.mirabilis,*respectively.

4- The control of these multidrug resistant organisms is atherapeutic challenge. This difficulty is enhanced further by theco-existence of the resistance to -lactams, aminoglycosides and quinolones as observed in ourstudy.

5- Of all the available antimicrobialagents, carbapenems are the most active especially Meropenamwas the highest effectiveantibiotic against all the isolated bacteria and reliabletreatment options for infections, which are caused by the ESBL producing isolates.

6-ESBL producers can be detected bytwo phenotypic methodswith equal efficacy. Thus, this study emphasizes the inclusion of ESBL detection in routine laboratory tests in hospital and clinics especially in the developing countries. Therefore, it is essential toreport ESBL production along with the routine sensitivity reporting,which will help the clinicians in prescribing proper antibiotics.

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