# Molecular Detection of Antibiotics Resistance Genes in *Burkholderia cepecia* isolated from Diabetic foot infection

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## Summary

This study aimed to isolate and diagnose *Burkholderia cepetia* from clinical specimens of diabetic foot and study the resistance of bacteria to antimicrobial agent in Najaf governorate from Augest 2019 to November 2019, which includes 120 clinical specimens for both sexes with an age ranged between )35-70) years old. The diagnosis of *bacteria* isolates was based on microscopy, as well as the culture and biochemical characteristics as an initial diagnosis. The final diagnosis by the Vitek-2 compact system .

*Burkholderia cepacia* 8(6.6%). Antibiotic sensitivity test was examined by dick diffution method, *Burkholderia cepacia* isolates showed high level of resistance almost for all β-lactam antibiotic classes under study which included;, ceftriaxone, cefoxitin and Cefepime, with percentage of (100%); ticarcllin with clavulalnic acide ,piperacllin , ceftazidime, tobramycine, ciproflaxcin and levofloxacin with percentage of (87.5%); aztreonam and amikacine with percentage(62.5); meropenem , imipenem and gentiamycin with percentage of (37.5%).

At molecular study, the investigated the presence of antibiotic-resistant genes (blaImp, blaOxa, blaKpc,blaCTX-M) using PCR technique and electrophoresis systems. 6/8 *Burkholderia cepacia* isolates were with *blaOXA* and (6/8) of isolates carry *blaCTX-M* gene, all isolates of *Burkholderia cepacia* gave negative result of blaIMP and blaKPC gene. Finally PCR analysis showed that the integron gene was (3/8 %).

***Keyword:Bcc,*** *Burkholderia cepacia ; Antibiotics Resistance; Dabetic*

## Introduction

The *Burkholderia cepacia* complex (Bcc) organisms are opportunistic nosocomial pathogens capable of causing severe disease in immunocompromised individuals. Bacteria frequently employ disparate mechanisms that act synergistically to achieve elevated resistance [(1).]

However, these data may overestimate the occurrence of resistance in *Burkholderia cepetia* organisms as the study was carried out on patient isolates solicited because they were in fact multidrug resistant. Despite this caveat, resistance patterns, both intrinsic and acquired, must not be discounted in these organisms .The often high-level acquired or intrinsic resistance of non-enteric bacteria such as *P. aeruginosa* and *Burkholderia* species is in no small part attributable to synergy between reduced penetration into and efflux from the cell [(2).]

Diabetes mellitus (DM) is a chronic disease caused by inherited and/or acquired deficiency in production of insulin by the pancreas, or by the ineffectiveness of the insulin produced. Such a deficiency results in increased concentrations of glucose in the blood, which in turn damage many of the body's systems, in particular the blood vessels and nerve and constitutes the most frequent diabetes-related cause of hospitalization [(3).]

Diabetic foot ulcers is one of the main causes of mortality and morbidity among people with diabetes. Its include an injury to all layers of skin, necrosis or gangrene that usually occur on the soles of the feet, as a result of peripheral neuropathy or peripheral arterial disease (PAD) in diabetes patients [(4).]

## Materials and Methods

**specimens collection and bacterial identification**

A total of 120 samples were collected from diabetic foot ulcer who attended different hospitals during the period from Augest 2019 to November 2019 in Al-Najaf provenance, sample collection include,collection 120 pus samples swab specimens from diabetic foot infection ulcer, The specimens were transported by sterile transport swabs to the department of bacteriology laboratory. Each specimen was inoculated using direct method of inoculation on culture of selective media namely MacConkey, Blood ,Mannitol agar then inoculated at 37°C for 18-24 hours [5].

**DNA Extraction**

Genomic DNA was extracted by using a commercial extraction system (Genomic DNA promega Kit ).

Molecular Identification

Gel electrophoresis was used for detection of DNA by UV transilluminator . The PCR assay was performed to detect the antibiotic resistance gene for *Burkholderia cepacia* shown in table(2). This primer was designed by Alpha DNA company, Canada as in table (1) . Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µLof 10mg/mL ethidium bromide (Sigma, USA) and it run at 80v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products [6].

**Table (1): Primers used in this study**

| Primer Type | Primer Target | Primer sequence (5'-3') | Amplicon size (bp) | Reference |
|---|---|---|---|---|
| CTX-M | $bla_{CTX-M}$ | **F**: SCS ATG TGC AGY ACC AGT AA<br>**R**: CCG CRA TAT GRT TGG TGG TG | 554 | (7) |
| KPC | $bla_{KPC}$ | **F**: ATG TCA CTG TAT CGC CGT CT<br>**R**: TTT TCA GAG CCT TAC TGC CC | 893 | (8) |
| IMP | $bla_{IMP}$ | **F:** TTGACACTCCATTTACDG<br>**R:** GATYGAGAATTAAGCCACYCT | 139 | (9) |
| *OXA* | *blaOxa* | **F:** GGCACCAGATTCAACTTTCAAG<br>**R:** GACCCCAAGTTTCCTGTAAGTG | 564 | (9) |

**Table (2): PCR program of *intI* primer that apply in the thermocycler**

| Gene | Initial denaturation | No. of cycles | Denaturation | Annealing | Extension | Final extension |
|---|---|---|---|---|---|---|
| *blaCtxm* | 94 Cº for 4min. | 35 | 94 Cº for 30Sec | 63Cº for 1 min | 72 Cº for 1min. | 72Cº for 5min. |
| *blaKp*C | 94 Cº for 5 min. | 35 | 94 Cº for 1min | 50Cº for 1min. | 72 Cº for 1 min | 72Cº for 10min. |
| *blaImp* | 94 Cº 10 min | 30 | 94 Cº 40Sec | 55 Cº for 40Sec | 72 Cº for 1 min | 72 Cº for 10 min |
| *blaOxa* | 94 Cº for 10 min | 30 | 94 Cº for 40Sec | 60 Cº for 40Sec | 72 Cº for 1min | 72 Cº for 5 min |

## Results and Discussion

This study was conducted on 120 specimens from dabetic foot suspected patients during the period from September 2019 to December 2019

**4.9.1.2 Molecular identification of antimicrobial drug resistance of *Pseudomonas aerginosae and Burkholderia cepacia***

**4.9.3.2.1.blaOXA**

The result showed that the *blaOXA* resistance gene was detected in 6/8 *Burkholderia cepacia* as in figure (1) .

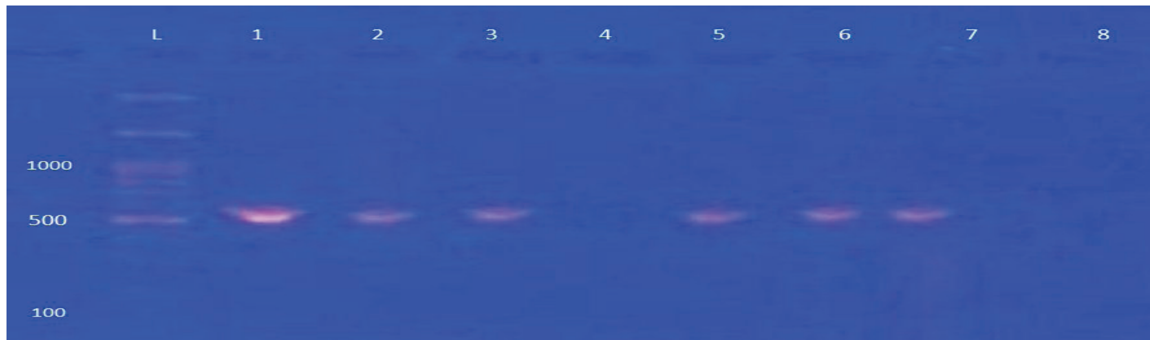


**Figure (1): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaOXA* gene primers with product 564bp.Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,5,6,7 ) show positive results with *blaOXA* gene.**

Carbapenemases are the main mechanism by which resistance to carbapenems occurs and they belong to three of the four β-lactamase classes A, B and D . Class D carbapenemases are the OXA-β-lactamases , further subdivided into various sub-groups mainly blaOXA-23, blaOXA-24/40, blaOXA-58, blaOXA-48, blaOXA-51 and blaOXA-143 . These OXA-type β-lactamases occur widely in Acinetobacter with the most abundant being blaOXA-51, which is chromosomally encoded hence intrinsic to these species but it may confer resistance to carbapenems when its expression is up-regulated by genetic re-organization [10] Class B carbapenemases are also known as the metallo-β-lactamases (MBLs) ,they are mostly encoded by integronborne mobile gene cassettes and hence, they are transferable amongst various bacteria via horizontal gene transfer mechanisms notably conjugation . Class A carbapenemases include the Klebsiella pneumoniae carbapenemase (KPC) family that can be plasmid encoded or chromosomal [11].

**4.9.3.2.2.blaIMP**

The result showed negative with *blaIMP* gene of *Burkholderia cepacia* isolates . Based on recent reports, there are two major families of imported metallo--lactamases, IMP and VIM, that are carried on mobile gene cassettes inserted into integrons . Including those in this report, there are 18 variants of IMP metallo--lactamases and 11 variants of VIM metallo--lactamases [12].

The only two published reports on metallo-B-lactamases from the United States identified VIM-2 and VIM-7. Metallo-B-lactamases hydrolyze most -lactam antibiotics except aztreonam. Therefore, many pathogens that produce these enzymes at high levels are resistant to the majority of -lactam antibiotics, including the carbapenems. The first report of an imported metallo-B-lactamase described a *Pseudomonas aeruginosa* isolate obtained from a Japanese patient in 1988 . Since then, the occurrence of mobile genetic elements encoding metallo-B-lactamases has extended beyond *P. aeruginosa* to include many types of gram-negative organisms distributed throughout the world . Areas which have reported these types of isolates include several countries in Asia and Europe; the Americas, including Brazil, Canada, and the United States; and Australia [12].

**4.9.3.2.3.blaKPC**

All *Burkholderia cepacia* isolates give negative result with *blaKPC* gene .

The blaKPC genes that encode KPCs are present on transferable plasmids and are flanked by transposable elements, thus allowing for the gene to move from plasmid to the bacterial chromosome and back . [13]. All the carbapenem resistant isolates showed 100% resistance to ampicillin, cotrimoxazole, all 4 generations of cephalosporins and piperacillin tazobactam. The resistance to aminoglycoside antibiotics varied from 33% for amikacin to 94% to tobramycin . In the present study, *Klebsiella* showed a 77% resistance to imipenem and 96% resistance to meropenem, while *E coli* showed 67% resistance to imipenem and 95% resistance to meropenem, blaKPC gene Detection in Clinical Isolates of Carbapenem Resistant Enterobacteriaceae were MHT negative. They may have developed a different resistant mechanism other than carbapenemase production. Resistant to both imipenem and meropenem is a strong indicator of carbapenemase production rather than resistance to either one of the carbapenems, as this may imply a different resistance mechanism [14].

**4.9.3.2.4.blaCTX-M gene**

The result showed that the blaCTX-M resistance gene was detected in and 6/8 *Burkholderia cepacia* as in figure,(2) .

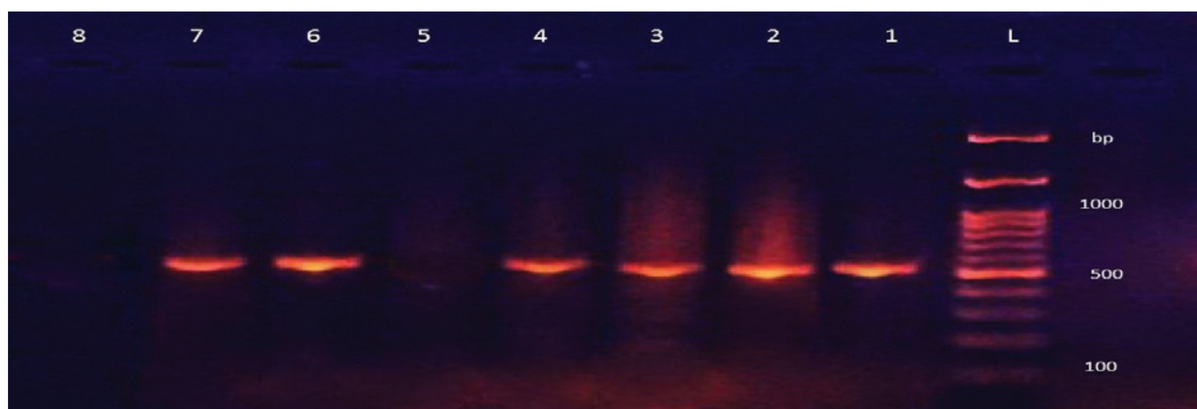


**Figure (2): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaCTX-M* gene primers with product 554bp.Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,4,6,7 ) show positive results with *blaCTX-M* gene.**

ESBLs are one of the main leading causes of resistance to ß-lactam antibiotics among Gram-negative bacteria . These enzymes are plasmid-encoded β-lactamases that mediate resistance to penicillins, first-, second- and third- generation cephalosporins, such as cefotaxime, ceftriaxone, and ceftazidime . TEM, SHV, and CTX-M are the major genetic groups of ESBLs amongst clinically important Gram-negative bacteria . These enzymes are most commonly found in Klebsiella pneumoniae (K. pneumoniae) and Escherichia coli (E. coli) and are also observed in other clinical isolates of Enterobacteriaceae and Pseudomonas [15]. The first TEM-type β-lactamase, produced by a clinical E. coli strain, was reported in 1965. The TEM-type ESBLs are derivatives of TEM-1 and TEM-2. The SHV-type ESBLs may be found in clinical isolates more frequently than any other types of ESBLs and have been reported from several countries in Europe, such as Austria, France, Italy, and Greece, as well as in the United States and Australia . The CTX-M-type ESBLs developed from TEM and SHV and can be divided into five subgroups according to their amino acid sequence simi-larities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V [16]

**Ethical Clearance:** The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

**Conflict of Interest:** Non

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