

# Molecular Detection of Antibiotics Resistance Genes in *Burkholderia Cepacia* Isolated From Diabetic Foot Infection

Maryam Riyadh Al-Muhanna<sup>1</sup>, Mahdi Al-Ammar<sup>1</sup>

<sup>1</sup>University of Kufa-Faculty of Science-Department of Biology/Iraq

## Summary

This study aimed to isolate and diagnose *Burkholderia cepacia* from clinical specimens of diabetic foot and study the resistance of bacteria to antimicrobial agent in Najaf governorate from August 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 diffusion method, *Burkholderia cepacia* isolates showed high level of resistance almost for all  $\beta$ -lactam antibiotic classes under study which included; ceftriaxone, ceftazidime and Cefepime, with percentage of (100%); ticarcillin with clavulanic acid, piperacillin, ceftazidime, tobramycin, ciprofloxacin and levofloxacin with percentage of (87.5%); aztreonam and amikacin with percentage(62.5); meropenem, imipenem and gentamicin 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; Diabetic

## 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 <sup>(1)</sup>.

However, these data may overestimate the occurrence of resistance in *Burkholderia cepacia* 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 <sup>(2)</sup>.

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 <sup>(3)</sup>.

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 <sup>(4)</sup>.

## Materials and Method

### specimens collection and bacterial identification

A total of 120 samples were collected from diabetic foot ulcer who attended different hospitals during the period from August 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 <sup>(5)</sup>.

**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 <sup>(6)</sup>.

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

Primer Type	Primer Target	Primer sequence (5'-3')	Amplicon size (bp)	Reference
CTX-M	<i>bla</i> <sub>CTX-M</sub>	F: SCS ATG TGC AGY ACC AGT AA R: CCG CRA TAT GRT TGG TGG TG	554	(7)
KPC	<i>bla</i> <sub>KPC</sub>	F: ATG TCA CTG TAT CGC CGT CT R: TTT TCA GAG CCT TAC TGC CC	893	(8)
IMP	<i>bla</i> <sub>IMP</sub>	F: TTGACACTCCATTTACDG R: GATYGAGAATTAAGCCACYCT	139	(9)
OXA	<i>bla</i> <sub>Oxa</sub>	F: GGCACCAGATTCAACTTTCAAG 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
<i>bla</i> <sub>Ctxm</sub>	94 C° for 4min.	35	94 C° for 30Sec	63C° for 1 min	72 C° for 1min.	72C° for 5min.
<i>bla</i> <sub>Kpc</sub>	94 C° for 5 min.	35	94 C° for 1min	50C° for 1min.	72 C° for 1 min	72C° for 10min.
<i>bla</i> <sub>Imp</sub>	94 C° 10 min	30	94 C° 40Sec	55 C° for 40Sec	72 C° for 1 min	72 C° for 10 min
<i>bla</i> <sub>Oxa</sub>	94 C° for 10 min	30	94 C° for 40Sec	60 C° for 40Sec	72 C° for 1min	72 C° for 5 min

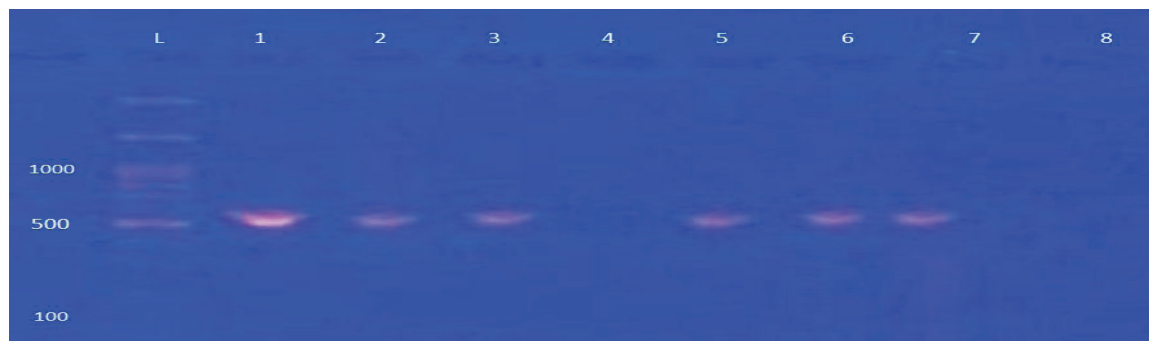
## Results and Discussion

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

### 4.9.1.2 Molecular identification of antimicrobial drug resistance of *Pseudomonas aeruginosa* 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  $\beta$ -lactamase classes A, B and D . Class D carbapenemases are the OXA- $\beta$ -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  $\beta$ -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 <sup>(10)</sup> Class B carbapenemases are also known as the metallo- $\beta$ -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 <sup>(11)</sup>.

#### 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 <sup>(12)</sup>.

#### 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 . <sup>(13)</sup> 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 <sup>(14)</sup>.

#### 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) .

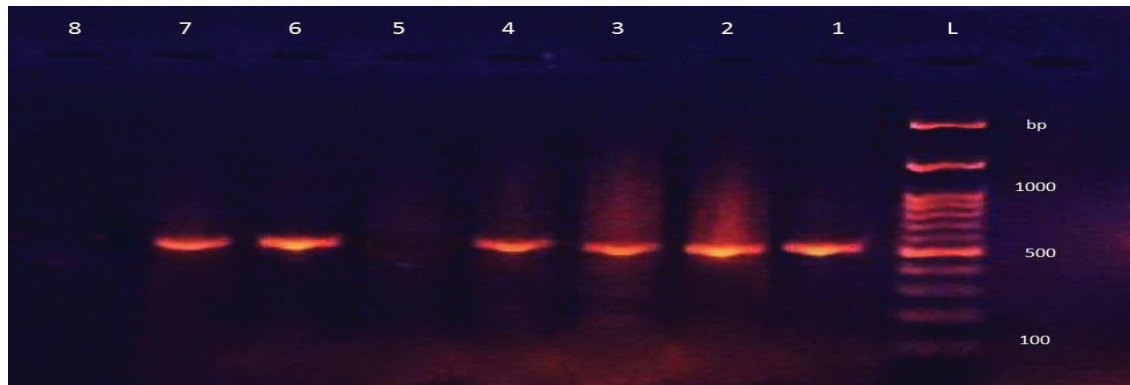


Figure (2): PCR amplification products of *Burkholderia cepacia* isolates that amplified with *blaCTX-M* gene primers with product 554bp. Lane (L), DNA molecular size marker (100-bp ladder), Lanes (1,2,3,4,6,7 ) show positive results with *blaCTX-M* gene.

ESBLs are one of the main leading causes of resistance to  $\beta$ -lactam antibiotics among Gram-negative bacteria . These enzymes are plasmid-encoded  $\beta$ -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* <sup>(15)</sup>. The first TEM-type  $\beta$ -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 similarities, including CTX-M-I, CTX-M-II, CTX-M-III, CTX-M-IV, and CTX-M-V <sup>(16)</sup>

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

**Conflict of Interest:** Non

**Funding:** Self-funding

#### References

- 1- **Kirketerp-Møller K, Jensen PØ, Fazli M, Madsen KG, Pedersen J, Moser C, Tolker-Nielsen T, Høiby N, Givskov M, Bjarnsholt T. Distribution, organization, and ecology of bacteria in chronic wounds. Journal of clinical microbiology.;** (2008). 46; 8; p2717-22.
- 2- **Danced. Treatment and prophylaxis of melioidosis. Intj Antimicrob Agents. (2014).43:310-318.**

- 3- **Spichler**, A.; Hurwitz, Bl.; Armstrong, D. And Lipsky, B. A. Microbiology Of Diabetic Foot Infections: From Louis Pasteur To ‘Crime Scene Investigation’. *Bmc Medicine*. (2015).
- 4- **Wang**, Ah.; Xu, Zr. And Ji, Ln. Clinical Characteristics And Medical Costs Of Diabetics With Amputation At Central Kirketerp-Møller Urban Hospitals In China. *Zhonghua Yi Xue Za Zhi*; (2012). 92:224-7.
- 5- **Chessbrough**, M. District Laboratory Practice in Tropical Countries, Part-2, New York, USA, Cambridge University, (2010). 184- 186.
- 6- **Levy**, H.; Diallo, S.; Tennant, SM.; Livio, S.; Sow ,SO.; Tapia, M.; Fields, PI.; Mikoleit, M.; Tamboura, B.; Kotloff ,KL.; Lagos, R.; Nataro, JP.; Galen, JE.and Levine, MM. PCR Method to Identify *Salmonella Enterica Serovars Typhi, Paratyphi A*, and *Paratyphi B* Among *Salmonella* Isolates from the Blood of Patients with Clinical Enteric Fever. *J Clin Microbiol*, (2008). 46(5):1861-1866.
- 7- **Saladin**,M.;Cao,V.T.;Lambert,T.;Donay,J.L.;Herrmann,J.L.;Ould-Hocine,Z.;Verdat,C.;Delisle,F.;Philippon,A.andArlet ,G. Diversity of CTX-M beta- lactamases and their promoter regions from Enterobacteriaceae isolated in three Parisian hospitals. *FEMS Microbiology Letters* , (2002). 209:161-168.
- 8- **Bradford**,P.A.;Bratus,s.;Urban,C.;Visalli,M. andMariano,N. Emergence of Carbapenem-Resistant *Klebsiella* Species possessing the class A Carbapenem-Hydrolyzing KPC-2 and Inhibitor – Resistant TEM30  $\beta$ -lactamases in New York City. *Clin. infectious Dis* ., (2004). 39:55-60.
- 9- **Dallenne**,C.,Dacosta,A.;Decre,D.;Favier,C. andArlet,G. Development of a set of Multiplex PCR assays for the detection of genes encoding important  $\beta$  –lactamases in Enterobacteriaceae .*J.ANTImicrob.Chemother.*, (2010). 65:490-495.
- 10- **Antunes** NT, Lamoureaux TL, Toth M, Stewart NK, Frase H, Vakulenko SB. Class D  $\beta$ -Lactamases: Are They All Carbapenemases? *Antimicrob Agents Chemother*. (2014). 58(4):2119–25.
- 11- **Manenzhe** RIZH, Nicol MP, Kaba M. The spread of carbapenemase producing bacteria in Africa: a systematic review. *J Antimicrob Chemother*. (2015).70(1):23–40.
- 12- **Walsh**, T. R., M. A. Toleman, L. Poirel, and P. Nordmann. Metallo-  $\beta$ -lactamases: the quiet before the storm? *Clin. Microbiol. Rev.* )2005(. 18:306–325.
- 13- **Nagaraj** S, Chandran SP, Shamanna P, Macaden R. Carbapenem resistance among *Escherichia coli* and *Klebsiella pneumoniae* in a tertiary care hospital in south India. *Indian J. Med. Microbiol*. (2012) . 30(1): 93-95.
- 14- **Francis RO**, Wu F, Phyllis DL, Shi J and Whittier S. Rapid Detection of *Klebsiella pneumoniae* Carbapenemase Genes in Enterobacteriaceae Directly From Blood Culture Bottles by Real-Time PCR. *Am J Clin Pathol*. (2012). 137(4):627-32.
- 15- **Adeyankinnu** FA, Motayo BO, Akinduti A, Akinbo J, Ogiogwa JI, Aboderin BW, Agunlejika RA. A multicenter study of betalactamase resistant *Escherichia coli* and *Klebsiella pneumoniae* reveals high level chromosome mediated extended spectrum  $\beta$ -lactamase resistance in Ogun state, Nigeria. (2014).
- 16- **Roschanski N**, Fischer J, Guerra B, Roesler U. Development of a multiplex real-time PCR for the rapid detection of the predominant beta-lactamase genes CTX-M, SHV, TEM and CIT type AmpCs in Enterobacteriaceae. *PLoS One* (2014). 9:e100956.