



## Prevalence of $\beta$ -lactamases in clinical isolates of *Enterobacter cloacae* in the West Bank-Palestine

\*Ghaleb Adwan<sup>1</sup>, Doa'a Rabaya<sup>2</sup>, Kamel Adwan<sup>1</sup> and Suhaila Al-Sheboul<sup>3</sup>

<sup>1</sup>Department of Biology and Biotechnology, An-Najah National University, P.O. Box (7)-Nablus, Palestine

<sup>2</sup>Department of Biological Science, Faculty of Graduate Studies, An-Najah National University, P.O. Box (7)-Nablus, Palestine

<sup>3</sup>Medical Laboratory Sciences Department, Jordan University of Science and Technology, P.O. Box (3030)-Irbid, Jordan

Corresponding e-mail: [adwang@najah.edu](mailto:adwang@najah.edu)

### ABSTRACT

The increasing spread of  $\beta$ -lactamase-producing pathogens represents an emerging serious public health threat specially to treat nosocomial infections. This study was carried out to determine the prevalence and molecular characterization of  $\beta$ -lactamase-producing *Enterobacter cloacae* isolates; and to estimate the prevalence of integrons in these isolates. A total of 41 clinical isolates of *E. cloacae* were recovered from different hospitals in the North West Bank-Palestine. *Enterobacter cloacae* isolates were identified using API 20E system and  $\beta$ -lactamase genes (ESBL, MBL and AmpC  $\beta$ -lactamase genes) detection was carried out using multiplex PCR technique. Results of the current research showed that the prevalence of  $\beta$ -lactamases among the studied clinical *E. cloacae* isolates was (34/41) 82.9%. The prevalence of ESBLs, MBLs and AmpC  $\beta$ -lactamase genes was 80.5%, 14.6% and 9.8%, respectively. For ESBL, *bla*<sub>TEM</sub> gene was the most dominant with a prevalence rate 63.4%. Other detected genes were 31.7%, 29.3, and 7.3% for *bla*<sub>OXA</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub>, respectively. Coexistence of 2 ESBL genes or more was detected in 39% of *E. cloacae* isolates. For AmpC  $\beta$ -lactamases only *bla*<sub>DHA</sub> gene was detected with a prevalence 4.9%, whereas for MBLs, the prevalence of *bla*<sub>IMP</sub> alone was 9.8%, *bla*<sub>SPM</sub> and *bla*<sub>IMP</sub>, and *bla*<sub>SIM</sub> and *bla*<sub>SPM</sub> together was 2.4% for each. A total of 8 isolates (19.5%) showed coexistence with at least another type of  $\beta$ -lactamases. In this study, class 1 integrons were detected only in  $\beta$ -lactamase-producing *E. cloacae* isolates with prevalence of (17/34) 50% among  $\beta$ -lactamase producers. ERIC-PCR typing of 34 clinical isolates of *E. cloacae* harbored different  $\beta$ -lactamase genes, were grouped into 5 ERIC PCR profiles (clusters) at a 70% similarity level. Results of ERIC-PCR typing showed that at least there are 3 identical clones circulating among these hospitals and the predominant clone is C1CL1. The emergence and increase of  $\beta$ -lactamase-producing *E. cloacae* infections is worrisome. Effective measures should be taken to control the spread  $\beta$ -lactamase-producing bacteria.

**Keywords:** *E. cloacae*, *Enterobacter* sp., ESBL, MBL, AmpC  $\beta$ -lactamase, Palestine.

### INTRODUCTION

*Enterobacter cloacae* (*E. cloacae*) is considered a significant hospital-acquired Gram-negative pathogen causing different infections including lower respiratory tract, urinary tract, skin and soft tissue, wounds, intravenous catheters, biliary tract, endocarditis, osteomyelitis, ophthalmic infections and central nervous system. This pathogen is intrinsically resistant to ampicillin and narrow spectrum cephalosporins owing to chromosomal cephalosporinase.<sup>[1,2]</sup> *Enterobacter* infections are increasing in frequency, specially in intensive care units (ICUs). In

the United States, *Enterobacter* was considered the fifth leading cause of ICU infections and third most common cause of nosocomial pneumonia overall, according to data collected between 1992 and 1999.<sup>[3]</sup>

Emergence of antimicrobial-resistant pathogenic microorganisms has become a worldwide health problem with serious consequences on the treatment of infectious diseases. It is clear that bacterial pathogens may become resistance to different classes of antimicrobial agents through the development of different intrinsic and acquired mechanisms.<sup>[4]</sup> A major mechanism for antibiotic resistance among Gram-negative bacteria is the production of  $\beta$ -lactamases. The most important  $\beta$ -lactamases are cephalosporinases like, extended spectrum  $\beta$ -lactamases (ESBLs) and the carbapenemases like metallo- $\beta$ -lactamases (MBLs).<sup>[5]</sup>

Recently, nosocomial outbreak of infections caused by *E. cloacae* producing  $\beta$ -lactamases were reported in some countries.<sup>[6-9]</sup> In Thessaloniki, Greece, 19 of 27 ceftazidime-resistant *E. cloacae* isolates from a neonatal intensive care unit had genes coding for the extended-spectrum  $\beta$ -lactamase IBC-1; 18 of those 19 harbored similar conjugative plasmids and belonged to two distinct genetic lineages.<sup>[6]</sup> In Spain, nosocomial outbreak in 2005 caused by ESBL-producing *E. cloacae* in a cardiothoracic intensive care unit (CT-ICU) was reported.<sup>[7]</sup> Genes detected in that outbreak were *bla*<sub>TEM</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M-9</sub> co-existed with *bla*<sub>SHV</sub>. In other study, twenty-one multiresistant *E. cloacae* isolates producing *bla*<sub>OXA-48</sub> (n=10), *bla*<sub>CTX-M-15</sub> (n=7) or both (n=4)  $\beta$ -lactamases were detected in a Spanish hospital during a 1-year period. Seven of these isolates belonging to clone 1 were carbapenem-resistant and carried the *bla*<sub>OXA-48</sub> gene, isolates of clone 2 (n=11) were resistant to cefepime and harboured the *bla*<sub>CTX-M-15</sub> gene. Four isolates belonging to clone 2 were also resistant to carbapenems owing to the co-production of *bla*<sub>OXA-48</sub>. Most of these isolates were recovered from patients admitted to intensive care units; a single patient was transferred from another Spanish hospital.<sup>[8]</sup> In recent study, the *Klebsiella pneumoniae* carbapenemase (*bla*<sub>KPC-2</sub>) gene was detected in 60% of outbreak of carbapenem-resistant *E. cloacae* in a pediatric intensive care unit of a teaching hospital in China, the *bla*<sub>IMP-8</sub> metallo- $\beta$ -lactamase gene was detected in 10% of the isolate. The New Delhi metallo- $\beta$ -lactamase (*bla*<sub>NDM-1</sub>) gene, was identified in 30% of the isolates. Furthermore, ESBLs and AmpC genes were detected in the majority of the carbapenem-resistant *E. cloacae* isolates.<sup>[9]</sup> In a university hospital in China, the prevalence of ESBL genes and carbapenemase genes among carbapenem-resistant *E. cloacae* isolates were 60.9% and 76.6%, respectively, and *bla*<sub>SHV-12</sub> and *bla*<sub>KPC-2</sub> were the most common genes, respectively.<sup>[10]</sup> High incidence and endemic spread of *bla*<sub>NDM-1</sub> carbapenem-producing *E. cloacae* isolates in Henan province, China was reported. About 73% of non-duplicated carbapenem-resistant *E. cloacae* isolates collected between June 2011 and May 2013 were identified as *bla*<sub>NDM-1</sub> positive.<sup>[11]</sup> In a Tertiary Care Centre in South India, it was shown that 76% of carbapenemase *Enterobacter* sp. producers carried *bla*<sub>NDM-1</sub> gene.<sup>[12]</sup>

In France, the prevalence of ESBLs among *E. aerogenes* and *E. cloacae* isolates was 53.5% and 6.7%, respectively. The frequency was 50% and 3.5% for *bla*<sub>TEM</sub> types and *bla*<sub>SHV-4</sub> genes among *E. aerogenes* isolates, while 3.3% for each *bla*<sub>TEM-3</sub> and *bla*<sub>CTX-M-3</sub> among *E. cloacae* isolates.<sup>[13]</sup> Study work carried out in Nigeria, reported the frequency of ESBL-producing *Enterobacter* sp. among the clinical samples collected from two hospitals was 20%.<sup>[14]</sup> A study conducted at University of Pittsburgh Medical Center, Pennsylvania reported 33.3% frequency of ESBL-producing *E. cloacae*.<sup>[15]</sup> In Algeria, the prevalence of ESBLs among *E. cloacae* isolates was 17.7% with frequency 7.8%, 6.4%, 2.8% for genes encoding *bla*<sub>CTX-M-15</sub>, *bla*<sub>CTX-M-3</sub>, *bla*<sub>SHV-12</sub> and *bla*<sub>VEB-1</sub>, respectively.<sup>[16]</sup> Other study in the same country, the prevalence of ESBLs among *E. cloacae* isolates was 46.4%. In this study, all ESBL-producing *E. cloacae* isolates had *bla*<sub>CTX-M</sub> gene. The prevalence of *bla*<sub>TEM</sub>, *bla*<sub>DHA</sub> and *bla*<sub>SHV</sub> genes were 38.5%, 30.8% and 7.75%, respectively, co-existing of 2 ESBL genes or more was detected in 69.2% ESBL-producing *E. cloacae*.<sup>[17]</sup> In Pakistan, the prevalence of ESBLs among *E. cloacae* isolates in different hospitals had a range from 14.93%-79%.<sup>[18-20]</sup> In India, the prevalence of ESBL- and MBL-producing isolates among *Enterobacter* sp. were 33.3% and 16.6%, respectively.<sup>[21]</sup> AmpC- and ESBL-producing *E. aerogenes* isolates recovered from pregnant woman was detected.<sup>[22]</sup> Extended-spectrum AmpC cephalosporinases have been reported in clinical isolates of *E. aerogenes*, which had hydrolysing activity against fourth-generation cephalosporins.<sup>[23]</sup>

Different  $\beta$ -lactamase genes were detected in *Enterobacter* sp.; *bla*<sub>VIM-4</sub> in *E. cloacae*,<sup>[24]</sup> *bla*<sub>IMP-4</sub> in *E. cloacae*,<sup>[25]</sup> *bla*<sub>KPC-3</sub> in *Enterobacter* sp.,<sup>[26]</sup> *bla*<sub>CTX-M-15</sub> in *E. cloacae*,<sup>[27]</sup> *bla*<sub>IMP-1</sub> in *E. cloacae* and in *E. aerogenes*,<sup>[28,29]</sup> *bla*<sub>KPC-2</sub>, *bla*<sub>DHA-1</sub> and *bla*<sub>TEM-1</sub> in *E. aerogenes*,<sup>[30]</sup> *bla*<sub>OXA-48</sub> in *E. cloacae*,<sup>[31]</sup> *bla*<sub>SHV-128</sub> type in *E. cloacae* isolate,<sup>[32]</sup> *bla*<sub>IMP</sub> in *E. aerogenes*,<sup>[33]</sup> *bla*<sub>KPC-2</sub> and *bla*<sub>NDM-1</sub> in *E. hormaechei* subs and in *E. cloacae*.<sup>[34,35]</sup>

Little or rare information is available about the prevalence and molecular characterization of  $\beta$ -lactamases in Palestine, no documented reports yet on the occurrence of  $\beta$ -actamase-producing *Enterobacter* species from this

country. The current study was conducted to determine the prevalence and molecular characterization  $\beta$ -lactamase-producing *E. cloacae* isolates using multiplex PCR technique and to assess the prevalence of class 1, 2 and 3 integrons in these isolates.

## MATERIALS AND METHODS

### *Sample collection and E. cloacae identification*

A total of 41 isolates of *E. cloacae* (7 blood, 15 urine, 2 sputum, 11 swab, 5 wound and 1 tissue) were isolated from patients (17 males and 24 females) at An-Najah Natinal University Hospital-Nablus (n=21), Rafidia Hospital-Nablus (n=2) and Al-Watani Hospital-Nablus (n=1), The Martyr Dr. Khalil. S. Hospital-Jenin (n=5), Al-Amal Hosptal-Jenin (n=1), AL-Razi Hospital-Jenin (n=3), Al-Shamal Lab-Jenin (n=3), Thabet Hospital-Tulkarem (n=5) during January-December 2015. All isolates were identified with API 20 E collections (BioMérieux, Marcy Étoile, France).

### *Antibacterial sensitivity test*

Antimicrobial sensitivity was determined according to the instructions described by the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method.<sup>[36]</sup> All *E. cloacae* isolates were examined using disks (Oxoid) to determine resistance against Meropenem (MEM) 10 $\mu$ g, Ceftazidime (CAZ) 30 $\mu$ g, Cefotaxime (CTX) 30 $\mu$ g, Aztreonam (ATM) 30 $\mu$ g, Imipenem (IMP) 10 $\mu$ g and Ceftriaxone (CRO) 30 $\mu$ g. Inhibition zones were measured and the *E. cloacae* isolates were classified as resistant or susceptible according to the criteria recommended by CLSI guidelines.<sup>[36]</sup>

### *DNA isolation*

Genome of *E. cloacae* was prepared for PCR according to the method described previously.<sup>[37]</sup> Briefly, cells were scraped off an overnight nutrient agar plate, washed twice with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), the pellet was resuspended in 0.4 ml of sterile distilled H<sub>2</sub>O, and boiled for 10-15 min. Then, cells were incubated on ice for 10 min. The debris was pelleted by centrifugation at 11,500 X g for 5 min. DNA concentration was measured using a spectrophotometer and the samples were stored at -20°C until use for further DNA analysis.

### *Amplification of MBL genes*

Detection of gene sequences coding for the VIM, IMP, SPM-1, GIM-1 and SIM-1 enzymes was performed by the multiplex PCR using oligonucleotide primer sets described previously.<sup>[38]</sup> Briefly, PCR reactions were performed in a final volume of 25  $\mu$ l of the amplification mixture containing 12.5  $\mu$ l of PCR premix with MgCl<sub>2</sub> (ReadyMix<sup>TM</sup> Taq PCR Reaction Mix with MgCl<sub>2</sub>, Sigma), 0.4  $\mu$ M of each primer, 3  $\mu$ l of DNA template. Genes amplification was carried out with a thermal cycler (Mastercycler Personal, Eppendorf) using the following conditions: 94°C for 5 min; 94°C for 30 sec, 52°C for 40 sec and 72°C for 50 s for 36 cycles; with a final extension at 72°C for 5 min. PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide.

### *Detection of KPC, NDM and DIM genes*

Detection of gene sequences coding for the *KPC*, *NDM* and *DIM* genes was performed by the multiplex PCR using oligonucleotide primer sets described previously.<sup>[39]</sup> PCR reactions and thermal conditions were performed as well as described in detection of MBL genes. PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide.

### *Detection of AmpC $\beta$ -lactamase genes*

All *E. cloacae* isolates were screened for the presence of AmpC gene sequences coding for the MOX, CMY, LAT, BIL, DHA, ACC, MIR-1T ACT-1, FOX enzymes. The oligonucleotide primer sets for these genes were described previously.<sup>[40]</sup> PCR reactions were performed as well as described in detection of MBL genes. Genes amplification was carried out with a thermal cycler (Mastercycler Personal, Eppendorf) using the following conditions: 94°C for 3 min; 94°C for 30 sec, 64°C for 30 sec and 72°C for 1 min for 25 cycles; with a final extension at 72°C for 5 min. PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide.

### *Detection of ESBL genes*

All *E. cloacae* isolates were screened for the presence of gene sequences coding for the TEM, SHV, CTX-M and OXA enzymes by multiplex PCR. The oligonucleotide primer sets for these genes were described previously; *bla*<sub>SHV</sub> genes,<sup>[41]</sup> *bla*<sub>TEM</sub> genes,<sup>[42]</sup> *bla*<sub>CTX-M</sub> genes,<sup>[43]</sup> and *bla*<sub>OXA</sub> genes.<sup>[44]</sup> PCR reactions were carried out as well as described in detection of MBL genes. Genes amplification was performed with a thermal cycler (Mastercycler

Personal, Eppendorf) using the following conditions: 94°C for 5 min; 94°C for 30 sec, 60°C for 30 sec and 72°C for 2 min for 25 cycles; with a final extension at 72°C for 5 min. PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide.

#### Detection of class 1, 2 and 3 integrons

All *E. cloacae* isolates were screened for the presence of integrase genes *int11*, *int12* and *int13* using primers previously described.<sup>[45]</sup> PCR reactions were performed as well as described in detection of MBL genes. The amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 3 min at 94°C followed by 35 cycles of denaturation at 94°C for 40 s; annealing at 58°C for 40 s; extension at 72°C for 40 s; with a final extension step at 72°C for 2 min. PCR products were visualized on a 1.5 % agarose gel stained with ethidium bromide.

#### ERIC-PCR typing

ERIC- (Enterobacterial repetitive intergenic consensus) PCR was performed using Primer ERIC1: 5'-ATG TAA GCT CCT GGG GAT TCA C-3' and Primer ERIC2: 5'-AAG TAA GTG ACT GGG GTG AGC G-3'. Each PCR reaction mix (25 µl) composed of 10 mM PCR buffer pH 8.3; 3 mM MgCl<sub>2</sub>; 0.4 mM of each dNTP; 0.8 µM of each primer; 1.5U of Taq DNA polymerase and 3 µl of DNA extract solution. DNA amplification was carried out using the thermal cycler (Mastercycler personal, Eppendorf, Germany) according to the following thermal conditions: initial denaturation for 2 min at 94°C was followed by 40 cycles of denaturation at 94°C for 50 s, annealing at 50°C for 40 s and extension at 72°C for 1 min, with a final extension step at 72°C for 5 min. The PCR products were analyzed by electrophoresis on 1.5% agarose gel. The gel images were scored using binary scoring system that recorded the absence and presence of bands as 0 and 1, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistical software version 20 (IBM). The number of different bands in each fingerprint was considered for comparison of bacterial species as previously described,<sup>[46]</sup> based on the following criteria: identical clones (no different band), "closely related clones" (have 1 different band), "possibility different clones" (have two different bands), "different clones" (have three or more different bands).

## RESULTS

#### Antibiotic susceptibility

Results of the current study showed that most of *E. cloacae* isolates were susceptible to Imepenem, while 95.1% and 68.3% of the isolates were resistant to Meropenem and Ceftriaxone, respectively. Results of antibiotic resistance of these isolates against antimicrobial agents are presented in Table 1.

#### Detection of β-lactamases and integrons

Using multiplex PCR technique, results of the current research showed that the prevalence of β-lactamases among the 41 studied clinical isolates of *E. cloacae* was (34/41) 82.9%. The prevalence of ESBLs, MBLs and AmpC β-lactamase genes was 80.5%, 14.6% and 9.8%, respectively. For ESBL genes, *bla*<sub>TEM</sub> gene was the most common with a prevalence 63.4%. Prevalence of *bla*<sub>OXA</sub>, *bla*<sub>SHV</sub> and *bla*<sub>CTX-M</sub> genes was 31.7%, 29.3 and 7.3%, respectively. Coexistence of 2 ESBL genes or more was detected in 39% of *E. cloacae* isolates. For AmpC β-lactamases only *bla*<sub>DHA</sub> gene was detected with a prevalence 4.9%, whereas for MBLs, the occurrence of *bla*<sub>IMP</sub> alone was 9.8%, *bla*<sub>SPM</sub> and *bla*<sub>IMP</sub>, and *bla*<sub>SIM</sub> and *bla*<sub>SPM</sub> together was 2.4% for each. In addition, eight isolates (19.5%) showed coexistence with at least another type of β-lactamases. Prevalence of β-lactamase genes are presented in Table 2 and Figure 1, 2 and 3.

In this study, class 1 integrons were detected only in β-lactamase-producing *E. cloacae* isolates with prevalence (17/34) 50% among β-lactamase producers. Results are presented in Figure 4.

#### ERIC-PCR analysis

ERIC-PCR typing of 34 clinical isolates of *E. cloacae* harbored different β-lactamase genes were grouped into 5 ERIC PCR profiles (clusters) at a 70% similarity level. Results of ERIC-PCR typing showed that at least there are 3 clones circulating among these hospitals and the predominant clone is C1CL1. Results of ERIC-PCR fingerprint are presented in Figures 5 and 6.

## DISCUSSION

Emergence of antimicrobial-resistant pathogenic microorganisms has become a worldwide health problem with serious consequences on the treatment of infectious diseases. Beta-lactam antibiotics are broadly used in the treatment of bacterial infections; this may lead a significant increase in the incidents of  $\beta$ -lactamase associated infections throughout the world.<sup>[47]</sup>

Results of current research showed that the prevalence of ESBL among *E. cloacae* in the North West Bank-Palestine is too high. The frequency of ESBL-producing *E. cloacae* strains varies in different studies from different countries.<sup>[17]</sup> Prevalence of ESBL-producing *Enterobacter* sp. ranged from 3.5%-79%.<sup>[13-21]</sup> Results of this study showed that *bla*<sub>TEM</sub> gene was most common in *E. cloacae* isolates, which is in agreement with previous study.<sup>[13]</sup> In contrast, other studies showed that *bla*<sub>CTX-M</sub>-type ESBL was most common in *Enterobacter* sp.<sup>[16,17]</sup> Coexistence of 2 ESBL genes or more was common in clinical isolates of *E. cloacae*. Presence of coexistence of ESBL genes in a single isolate was previously reported.<sup>[7,8,16,17]</sup>

Plasmid-encoded AmpC cephalosporinases closely related in sequence to chromosomal AmpC enzymes from several members of the family *Enterobacteriaceae*.<sup>[48]</sup> These enzymes are often found in most *Enterobacteriaceae* as chromosomal enzymes. The transfer of AmpC genes to plasmids has resulted in their dissemination among *Enterobacteriaceae*, with the consequence that AmpC-encoded  $\beta$ -lactamases are now present in strains of different species belonged to *Enterobacteriaceae*. Evidence that transfer of a *bla*-coding plasmid from *K. pneumoniae* to *E. cloacae* had probably occurred in vivo.<sup>[24]</sup> Plasmid-mediated AmpC enzymes have been described from diverse geographic areas.<sup>[49]</sup> Phenotypic tests are not able to differentiate between chromosomal AmpC genes and AmpC genes that are carried on plasmids. A multiplex PCR for six families of plasmid-carried AmpC genes may be used to detect the presence of these externally acquired AmpC genes.<sup>[40]</sup> Results of this study showed that 4.9% of *E. cloacae* isolates carried AmpC  $\beta$ -lactamase genes. This low prevalence of AmpC  $\beta$ -lactamases among *Enterobacter cloacae* isolates was due to that primers in this research used to detect plasmid encoding genes only, while conventional methods have ability to detect both plasmid and non-plasmid-derived (chromosomal) AmpC activity.<sup>[50,51]</sup> Extended-spectrum AmpC cephalosporinases (ESACs) have been reported in clinical isolated of *E. aerogenes*, which had hydrolysing activity against fourth-generation cephalosporins.<sup>[23]</sup> AmpC  $\beta$ -lactamase *bla*<sub>DHA-1</sub> type has been detected in *E. aerogenes* and in carbapenem-resistant *E. cloacae*.<sup>[9,30]</sup>

Our results showed that the prevalence of MBL among clinical *E. cloacae* isolates was 14.6%. This result is in agreement with a recent study, which showed that the prevalence of MBL-producing isolates among *Enterobacter* sp. was 16.6%.<sup>[21]</sup> The occurrence of *bla*<sub>IMP</sub> alone was 9.8%, while *bla*<sub>SPM</sub> and *bla*<sub>IMP</sub>, and *bla*<sub>SIM</sub> and *bla*<sub>SPM</sub> together was 2.4% for each. However, these results were in contrast to other studies, which showed that 73%-76% of carbapenemase *Enterobacter* sp. producers carried *bla*<sub>NDM-1</sub> gene.<sup>[11,12]</sup> In other study, they showed that *bla*<sub>KPC-2</sub> and *bla*<sub>NDM-1</sub> genes were detected in 60% and 30% of carbapenem-resistant *E. cloacae*, respectively.<sup>[9]</sup> In other studies, *bla*<sub>IMP</sub> or its types was detected in *Enterobacter* sp. such as *bla*<sub>IMP-1</sub> in *E. cloacae* and in *E. aerogenes*,<sup>[28,29]</sup> *bla*<sub>IMP-4</sub> in *E. cloacae*,<sup>[25]</sup> *bla*<sub>IMP</sub> in *E. aerogenes*.<sup>[33]</sup>

Coexistence of more than one of  $\beta$ -lactamase classes or multiple genes of ESBLs, MBLs or AmpC  $\beta$ -lactamases has been reported from different species of bacterial pathogens including clinical *E. cloacae* isolates.<sup>[9,10]</sup> Coexistence of two or three types of  $\beta$ -lactamase genes in single isolates was observed in the current study among *E. cloacae*, this may seriously restrict the options of treatment, create diagnostic challenge and may lead to use an unsuitable antimicrobial therapy and the result may be fatal.<sup>[52]</sup>

In this study, 50% of  $\beta$ -lactamase-producing *E. cloacae* isolates carried class 1 integrons and none carried other tested classes of integrons. This result is in agreement with other report recently published from the same region, which showed that the class 1 integrons was the only type detected among clinical isolates of  $\beta$ -lactamase-producing *K. pneumoniae* with the prevalence (26/48) 45.25%.<sup>[51]</sup> High prevalence of class 1 integrons supports and rapid dissemination of  $\beta$ -lactamase genes and other multiple antibiotic resistance determinants among bacterial species in hospitals.<sup>[53]</sup> Intrahospital and interhospital dissemination of multiresistant *E. cloacae* isolates is of major clinical concern as it could lead to endemic nosocomial situations.<sup>[8]</sup>

ERIC-PCR typing of 34  $\beta$ -lactamase-producing *E. cloacae* isolates a 70% similarity cut-off value analysis showed, that these isolates were divided into 5 clusters. At least 3 clones were circulating among these hospitals and the

predominant clone is C1CL1. These infections may be due to clone dissemination and may be horizontal spread coexisted in this outbreak of nosocomial infections. The high prevalence of  $\beta$ -lactamase-producing *E. cloacae* isolates may due to selective pressure resulting from uncontrolled, extensive incorrect and misuse of these antibacterial agents especially cephalosporins in hospitals as well as in the country as a whole.<sup>[50,51]</sup>

Table 1. Antibiotic resistance of 41 *E. cloacae* isolates recovered from different clinical samples

| Antibiotic  | Resistant strains |       |
|-------------|-------------------|-------|
|             | No.               | %     |
| Ceftriaxone | 28                | 68.3  |
| Cefotaxime  | 26                | 63.41 |
| Imipenem    | 5                 | 12.2  |
| Ceftazidime | 17                | 41.46 |
| Aztreonam   | 19                | 46.34 |
| Meropenem   | 39                | 95.1  |

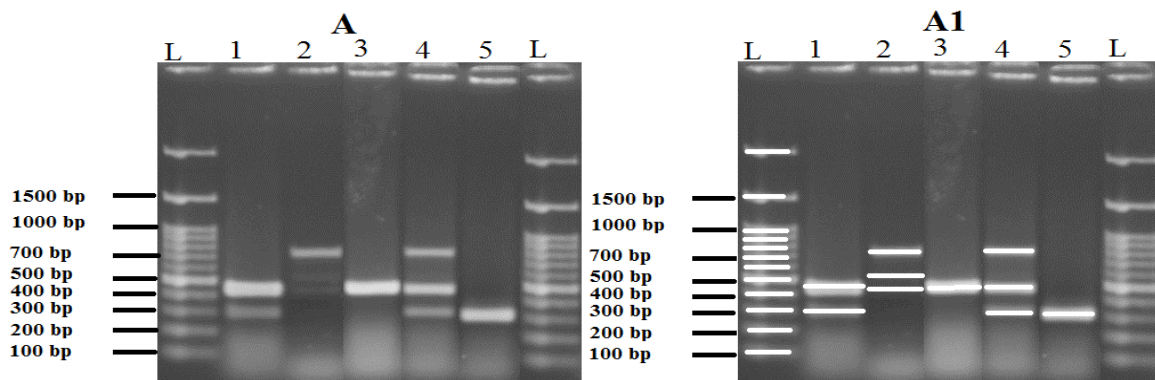


Figure 1. A: Multiplex PCR profiles specific for ESBL genes detected in clinical isolates of *E. cloacae*. L represents the ladder, other lanes for detected ESBL genes; *bla*<sub>TEM</sub> (445 bp), *bla*<sub>SHV</sub> (747 bp), *bla*<sub>OXA</sub> (296 bp) and *bla*<sub>CTX-M</sub> (593 bp)

Figure 1. A1: It is the same as A but bands are demarcated to be obvious

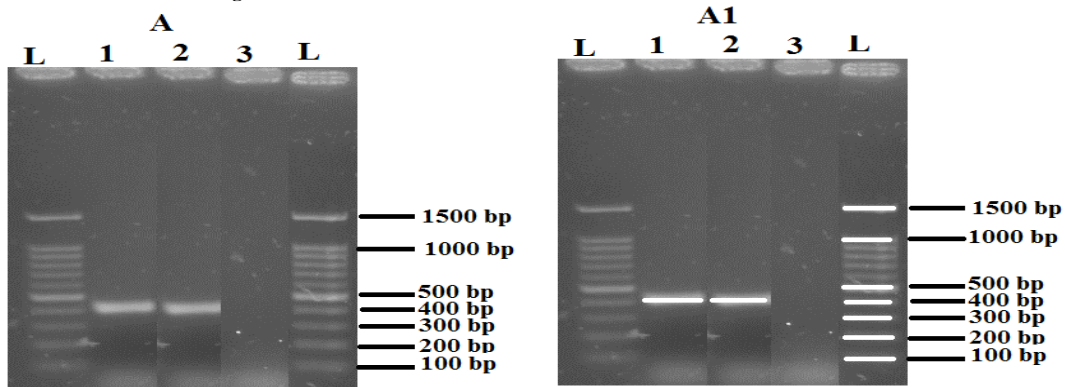


Figure 2. A: Multiplex PCR profiles specific for or AmpC  $\beta$ -lactamase genes detected in clinical isolates of *E. cloacae*.

L represents the ladder, lanes 1 and 2 represent *bla*<sub>DHA</sub> gene positive (405 bp)

Figure 2. A1: It is the same as A but bands are demarcated to be obvious

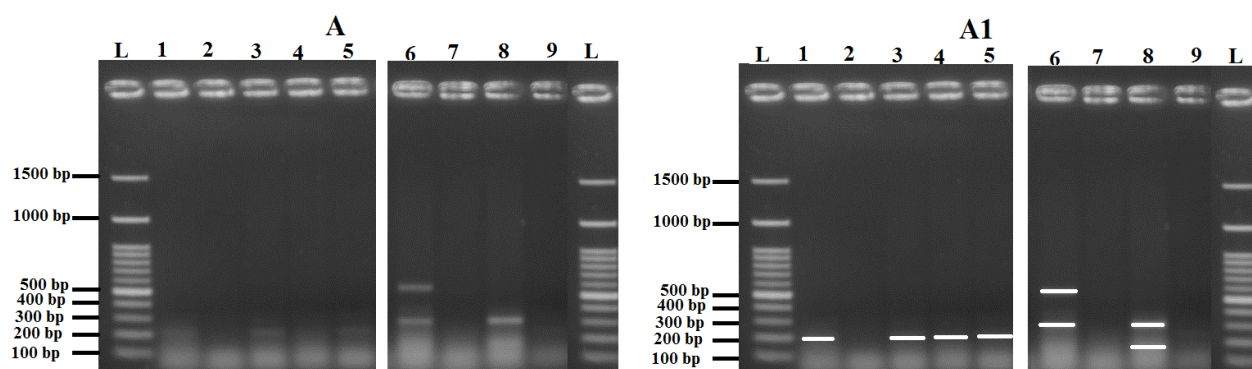


Figure 3. A: Multiplex PCR profiles specific for MBL genes detected in clinical isolates of *E. cloacae*. L represents the ladder, other lanes for detected MBL genes; *bla*<sub>IMP</sub> (188 bp), *bla*<sub>SPM</sub> (271 bp), *bla*<sub>SIM</sub> (570 bp)  
 Figure 3. A1: It is the same as A but bands are demarcated to be obvious.

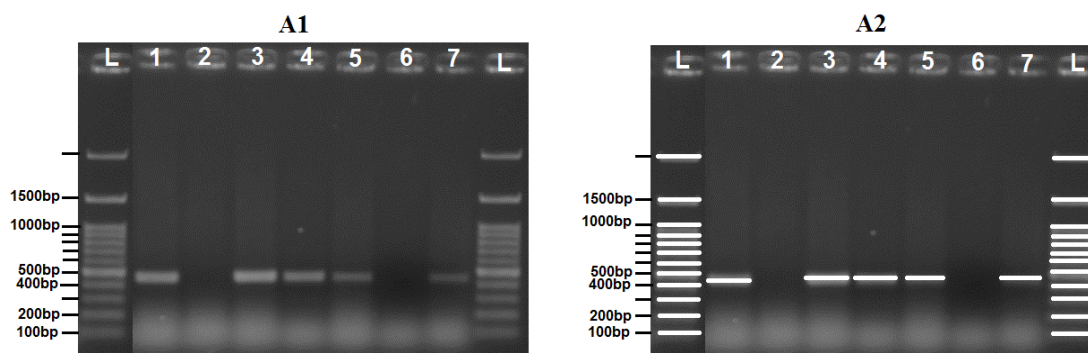


Figure 4. A: Multiplex PCR profile specific for integrons in clinical isolates of *E. cloacae*. L represents the ladder, Lanes 1, 3, 4 and 5 represent class I integron, Lanes 2 and 6 represent negative control and negative sample.  
 Figure 4. A1: It is the same as A but bands are demarcated to be obvious.

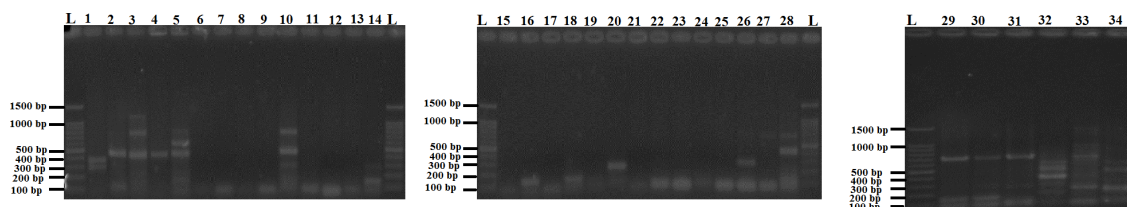


Figure 5. ERIC PCR profiles of 34 clinical *E. cloacae* isolates carried  $\beta$ -lactamase genes. Lanes L represent the ladder

Table 2. Prevalence of  $\beta$ -lactamase genes among 41 clinical isolates of *E. cloacae* detected by multiplex PCR technique

| $\beta$ -Lactamases                              |            |            |                           |                           |                                |                                  |                                 |                           |                           |  |
|--|------------|------------|---------------------------|---------------------------|--------------------------------|----------------------------------|---------------------------------|---------------------------|---------------------------|--|
| Extended spectrum $\beta$ -lactamases<br>No. (%) |            |            |                           |                           |                                |                                  | Metallo-B-lactamases<br>No. (%) |                           |                           | AmpC $\beta$ -<br>lactamase<br>No. (%) |
| Class (A)  |            | Class (D)  |                           |                           |                                |                                  | Class (B)                       |                           |                           | Class (C)                              |
| <i>TEM</i>                                       | <i>SHV</i> | <i>OXA</i> | <i>TEM</i> and <i>OXA</i> | <i>TEM</i> and <i>SHV</i> | <i>TEM, SHV</i> and <i>OXA</i> | <i>TEM, CTX-M</i> and <i>SHV</i> | <i>IMP</i>                      | <i>SPM</i> and <i>IMP</i> | <i>SIM</i> and <i>SPM</i> | <i>DHA</i>                             |
| 10 (24.39)                                       | 2(4.9)     | 5 (12.2)   | 6 (14.6)                  | 5 (12.2)                  | 2 (4.9)                        | 3 (7.3)                          | 4 (9.8)                         | 1 (2.4)                   | 1 (2.4)                   | 2 (4.9)                                |
| Total 33 (80.48)                                 |            |            |                           |                           |                                |                                  | Total 6 (14.6)                  |                           |                           | Total 2 (4.9)                          |



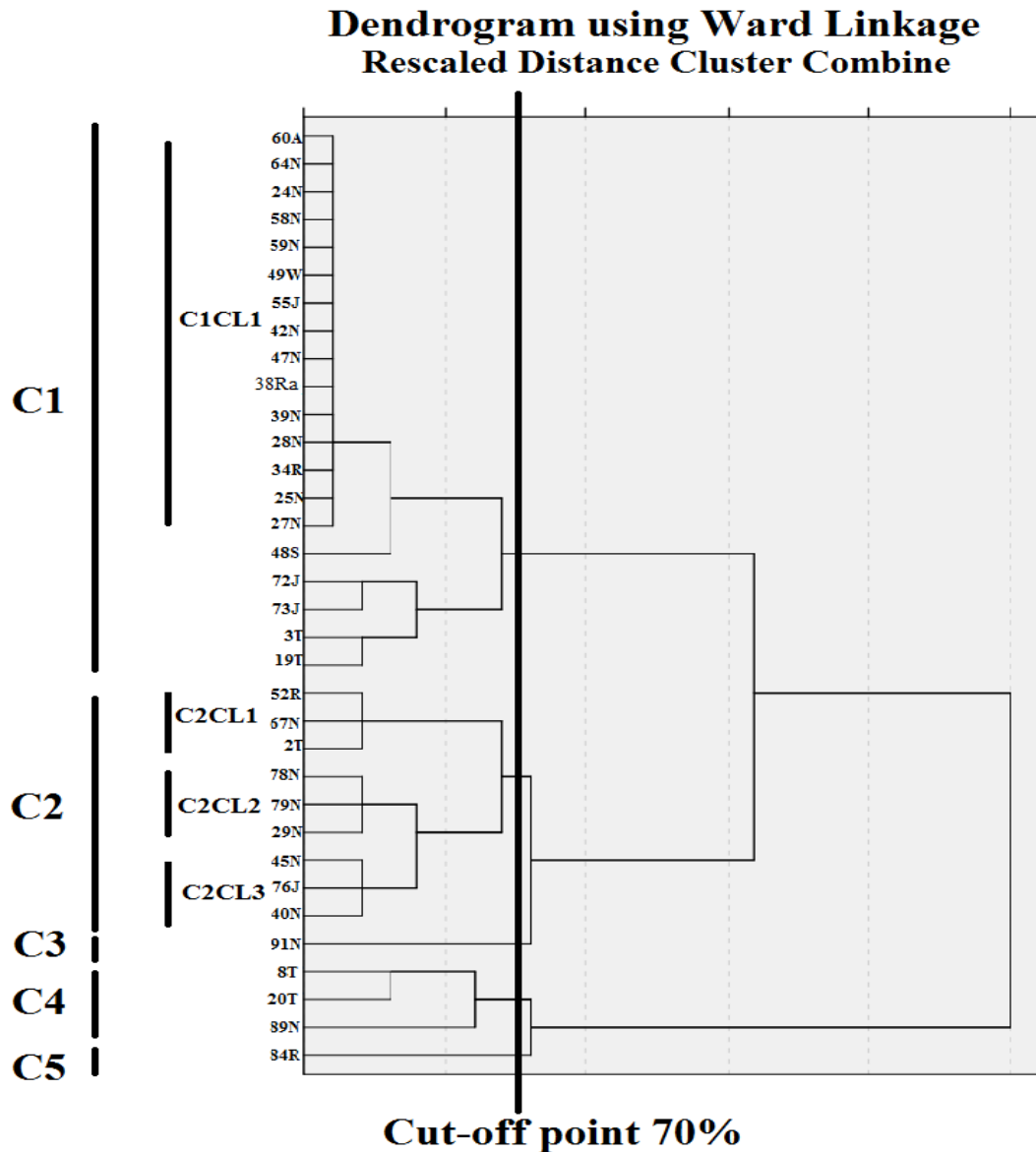


Figure 6. Dendrogram of 34 *E. cloacae* isolates carried  $\beta$ -lactamase genes based on the UPGMA method derived from analysis of the ERIC-PCR-profiles at a 70% similarity level.  
 C: Cluster, CL: clone, A: Amal Hospital, N: An-Najah Natinal University Hospital, W: Al-Watani Hospital, J: The Martyar Dr. Khalil. S. Hospital, Ra: Rafidia Hospital, R: AL-Razi Hospital, S: Al-Shamal Lab.

**CONCLUSION**

This is the first study documented the prevalence and molecular characterization of  $\beta$ -lactamase-producing *E. cloacae* isolates in West Bank-Palestine. The emergence and increase of  $\beta$ -lactamase-producing *E. cloacae* infections is worrisome. Effective measures should be taken to control the spread  $\beta$ -lactamase-producing bacteria. Correct diagnosis of  $\beta$ -lactamase-producing pathogen in due time is obligatory for optimal patient management and for immediate institution of appropriate infection control measures to prevent the spread of these organisms.



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