An-Najah National University

Faculty of Graduate Studies

Prevalence and Molecular Characterization of β-Lactamases in Clinical Isolates of *Klebsiella pneumoniae* from North of Palestine

By

Doa'a Faisal Rabaya'

Supervisor

Dr. Ghaleb Adwan

This Thesis is Submitted in Partial Fulfillment of the Requirements for The Degree of Master of Life Science (Biology), Faculty of Graduate Studies, An-Najah National University, Nablus, Palestine.

2016

Prevalence and Molecular Characterization of β-Lactamases in Clinical Isolates of *Klebsiella pneumoniae* from North of Palestine

By

Doa'a Faisal Rabaya'

This thesis was defended successfully on 5/4/2016 and approved by:

Defense Committee Members	**Signature**
– **Dr. Ghaleb Adwan / Supervisor**	.....
– **Dr. Mohammad Farraj / External Examiner**	.....
– **Dr. Motasem Al-Masri / Internal Examiner**	.....

Dedication

To the laurel crown that I wear…. My father's soul – God's mercy upon him.

To the candle that burns in order to illuminate my life…. My affectionate mother.

To the angelic companion who support me in this life…. My dear husband.

To that who got bored of being away from and whom I was always busy from…. My beloved daughter.

To the flowers that spread their perfume in my life…. My brother and sisters.

Acknowledgements

Praise and thanks be to God almighty, and peace and blessings be upon the messenger of guidance, peace be upon him.

I extend my great thanks and gratitude to my advisor Dr. Ghaleb Adwan who supervised my thesis and spared neither effort nor time in guiding and advising me.

I appreciate my husband's effort , Mr. Husam Rabaya, for giving technical assistance and guidance to complete my work.

I will not forget to thank all who made the lab environment friendly for working, the technicians in Department of Biology and Biotechnology at An-Najah National University for their help, support and cooperation. My thanks also go to those working in Microbiology labs in hospitals (An-Najah National University Hospital, Rafidia Hospital, al-Watani (the National) Hospital, The Martyar -Dr. Khalil S. Hospital, Al-Amal Hospital, AL-Razi Hospital, Thabat hospital and Al-Shamal Lab) for their help in sample collection.

At last, I would like to thank everyone who encouraged, advised and helped me get my work done.

الإقرار

أنا الموقعة أدناه، مقدمة الرسالة التي تحمل العنوان:

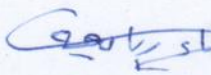
Prevalence and Molecular Characterization of β-Lactamases in Clinical Isolates of *Klebsiella pneumoniae* from North of Palestine

أقر بأن ما شملته هذه الرسالة إنّما هو نتاج جهدي الخاص، باستثناء ما تمّت الإشارة إليه حيثما ورد، وأنّ هذه الرسالة ككل، أو أيّ جزء منها لم يقدّم من قبل لنيل أيّ درجة أو لقب علميّ لدى أيّ مؤسسة تعليمية أو بحثية أخرى.

Declaration

The work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's Name: **اسم الطالبة: دعاء فيصل ربايعة**

Signature: **التوقيع:** 

Date: **التاريخ: 2016/4/5**

Table of Contents

List of Tables

List of Figures

List of abbreviations

ESBL	Extended-spectrum β-lactamase
MBL	Metallo- β-lactamase
MOX	Moxalactam
OXA	Oxacillin hydrolyzing capabilities
SHV	Sulfhydryl Variable
TEM	Temoneira
ACC	Ambler Class C
ACT	AmpC type
CTX-M	Cefotaxime hydrolyzing capabilities
DHA	Dhahran Hospital
FOX	Cefoxitin
PCR	Polymerase Chain Reaction
NPHL	National Public Health Laboratory
bla	β-lactamase
CLSI	Clinical and Laboratory Standards Institute
ERIC	Enterobacterial Repetitive Intergenic Consensus
SIM	Sulfide Indole Motility
MHA	Mueller Hinton agar
NA	Nutrient Agar
IMViC	Indole production, Methyl red test, Voges-Proskauer test and Citrate test
CRO	Ceftriaxone
CTX	Cefotaxime
CAZ	Ceftazidime
UPGMA	Unweighted Pair Group Method for Arithmetic averages
SPSS	Statistical Package for the Social Sciences
NCBI	National Center for Biotechnology Information
H_2S	Hydrogen Sulfide
$MgCl_2$	Magnesium Chloride

Prevalence and Molecular Characterization of β-lactamases in Clinical Isolates of *Klebsiella pneumoniae* from North Palestine

By
Doa'a Faisal Rabaya'
Supervisor
Dr. Ghaleb Adwan

Abstract

Fifty-one clinical isolates of *Klebsiella pneumoniae* were obtained from different hospitals in Jenin, Nablus and Tulkarem districts-Palestine, during September - December 2015. Identification of isolates were confirmed in Microbiology laboratories at An-Najah National University-Nablus, Palesine; using different tests such as growth on MacConkey, detection of growth at 5°C and 44.5°C, Gram stain reaction, motility test and other biochemical tests were used such as Indole test and Voges-Proskauer test. Multiplex PCR was used to detect and determine the molecular epidemiology of MBL, ESBL and AmpC β-lactamase producing *K. pneumoniae* isolates. Results showed that the prevalence of possible ESBL, MBL and AmpC β-lactamase using multiplex PCR technique were 92.2%, 9.8 and 3.9%, respectively. *TEM* gene was the most dominant (72.5%) among the *K. pneumoniae* isolates. Other genes were (0.0%), (17.6%) and (31%) for *CTX-M*, *SHV* and *OXA* genes, respectively. For MBLs and AmpC β-lactamases (9.8%) *NDM* and (3.9%) *DHA* genes were detected, respectively, among the collected isolates. Six isolates (11.8%) showed coexistence with at least another type of β-lactamases. Molecular analysis and phylogenetic relationships showed that all sequenced *TEM*, *SHV*, *OXA*, *NDM* and *DHA* genes belonged to *TEM-1*, *SHV-1*, *OXA-1*, *NDM-1* and

DHA-1, respectively. Results of the current study showed that all *K. pneumoniae* isolates were sensitive to Imipenem (100%), while 68.6% and 54.9% of the isolates were resistant to Meropenem and Cefotaxime, respectively. A total of 26 of *K. pneumoniae* isolates (51%) harbored class 1 integrons, whereas other classes were not detected. All integrons were detected in *K. pneumoniae* isolates carrying β-lactamase genes.

ERIC-PCR typing of 40 clinical isolates of *K. pneumoniae* harbored β-lactamase genes were genetically diverse and consisted of a heterogeneous population with a total of 16 ERIC PCR profiles (clusters) at a 50% similarity level.

Results of the current research showed high occurrence of β-lactamases among clinical isolates of *K. pneumoniae* in Palestine. To restrict the further spread of β-lactamases producing *K. pneumoniae* isolates within hospitals in this country, we recommend the continuous monitoring and surveillance of the prevalence, proper prevention practices and effective antimicrobial drugs use.

Chapter One

Introduction

1.1. General background

Human pathogens include *K. pneumoniae* subspecies *pneumoniae, ozaenae,* and *rhinoscleromatis; K. oxytoca; K. granulomatis; K. variicola;* and *K. singaporensis*. *K. planticola, K. terrigena*, and *K. orinthinolytica* have been transferred to the genus *Raoultella* (Janda and Abbott, 2006; Abbott, 2007). *Klebsiella* spp. are Gram-negative, nonmotile, usually encapsulated rod-shaped bacteria, belonging to the family *Enterobacteriaceae*. These bacteria produce lysine decarboxylase but not ornithine decarboxylase and are generally positive in the Voges-Proskauer test. They are generally facultative anaerobic, and range from 0.3 to 1.0 mm in width and 0.6 to 6.0 mm in length. *Klebsiella* spp. often occur in mucoid colonies (Janda and Abbott, 2006; Abbott, 2007).

Klebsiella spp. are common opportunistic pathogens of nosocomial infections which is considered as one of the leading causes of human morbidity and mortality (Podschun and Ullmann, 1998; Struve and Krogfelt, 2004). They have been identified as important common pathogens for nosocomial pneumonia (7 to 14% of all cases), septicaemia (4 to 15%), urinary tract infection (6 to 17%), wound infections (2 to 4%), intensive care unit (ICU) infections (4 to 17%), and neonatal septicaemias (3 to 20%). *Klebsiella* spp. can also cause bacteremias, hepatic infections and have been isolated from a number of unusual infection, including endocarditis, primary gas-containing mediastinal abscess, peritonitis, acute cholecystitis, crepitant myonecrosis, pyomyositis, necrotizing fasciitis, psoas muscle abscess, facial space infections of the head and neck, and

septic arthritis. They are also important opportunistic pathogens, particularly among the immunocompromised patients. Pathogenicity factors of *Klebsiella* spp. include adhesins, siderophores, capsular polysaccharides (CPLs), cell surface lipopolysaccharides (LPSs), and toxins, each of which plays a specific role in the pathogenesis of these species. Depending on the type of infection and the mode of infectivity, bacterial cells of *Klebsiella* spp. may adhere and attack upper respiratory tract epithelial cells, gastrointestinal tract cells, endothelial cells, or uroepithelial cells, followed by colonization of mucosal membranes. Common underlying conditions include alcoholism, diabetes mellitus, chronic liver disease (cirrhosis), chronic renal failure, cancer, transplants, burns, and/or use of catheters (Janda and Abbott, 2006).

Antimicrobial resistance has been identified as a key public health challenge and is often difficult to control (Spellberg *et al*., 2008). It is clear that bacteria will continue to develop resistance to currently available antibacterial agents and susceptible bacteria may become resistance to multiple classes of antimicrobial drugs through the development of intrinsic mechanisms to many antibiotics and acquired mechanisms, which have the ability to develop resistance through mutations in different chromosomal loci or through the horizontal acquisition of resistant genes, which are carried on plasmids, transposons or integrons (Hill *et al*., 2007, Hammami *et al*., 2011). A major mechanism for antibiotic resistance among Gram-negative bacteria is the production of β-lactamases. Beta-lactamases are commonly classified according to two general classification systems: the

Ambler molecular classification and the Bush–Jacoby–Medeiros functional classification (Ambler, 1980; Bush and Jacoby, 2010). The scheme classifies β-lactamases into four classes according to protein homology of enzymes. Beta-lactamases of class A, C, and D possess a serine active site called serine β-lactamase, whereas class B beta-lactamases are metalloenzymes usually requiring a zinc molecule for their catalytic activities. The Bush–Jacoby–Medeiros classification system groups β-lactamases according to functional properties; this classification system uses substrate and inhibitor profiles in an attempt to organize the enzymes in ways that can be correlated with their phenotype in clinical isolates (Bush and Jacoby, 2010). This classification grouped the beta-lactamases in 3 major groups and 16 subgroups. The most important beta-lactamases are cephalosporinases like, extended spectrum beta-lactamases (ESBLs) and the carbapenemases like metallo-beta-lactamases (MBLs) (Peterson, 2006). Class C cephalosporinases are among the most abundant β-lactamases on the basis of the number of organisms that produce these enzymes. These cephalosporinases, frequently named as species-specific AmpC enzymes, are often found in most *Enterobacteriaceae* as chromosomal enzymes. These enzymes are generally present at a low (basal) level but may be induced to high levels in the presence of selected inducing agents such as amoxicillin or clavulanic acid (Bush, 2010). Treatment of organisms producing an inducible AmpC cephalosporinase has created some controversy. A group of investigators has recommended that any AmpC-inducible *Enterobacteriaceae* be regarded as resistant to all third-

generation cephalosporins (Livermore *et al*., 2004). Plasmid-encoded AmpC cephalosporinases closely related in sequence to chromosomal AmpC enzymes from *Enterobacter cloacae*, *Citrobacter freundii*, or *Aeromonas* spp. also appear in *Enterobacteriaceae* in organisms that produce at least one other β-lactamase (Jacoby, 2009). Although some of the plasmid-encoded AmpC enzymes are inducible, most of the enzymes are produced at much higher levels than seen for basal AmpC cephalosporinases, similar to isolates with derepressed AmpC enzymes (Jacoby, 2009).

Extended-spectrum β-lactamases (ESBL) are enzymes produced by many Gram-negative bacteria, which have ability to change the susceptibility of different antimicrobial agents (Al-Muharrmi *et al*., 2008). These enzymes have the capability to hydrolyze and inactivate broad spectrum of β-Lactam antimicrobials, including third-generation cephalosporins, penicillins and aztreonam; but are inhibited by β-lactamase inhibitors such as clavulanic acid (Nathisuwan *et al*., 2001; Al-Muharrmi *et al*., 2008). In addition, ESBL-producing organisms are often able to reduce the susceptibility of other non-β-lactamase antimicrobial classes, such as aminoglycosides, sulphonamides, trimethoprim, tetracyclines, chloramphenicol, fluoroquinolones, and nitrofurantoin (Paterson, 2000; Winokur *et al*., 2001; Wang *et al*., 2004; Mammeri *et al*., 2005). Thus, very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing enterobacterial isolates. As a result,

ESBL-producing organisms pose a major problem for clinical therapeutics due to leaving a limited range of therapeutic agents.

Enterobacteriaceae, especially *Klebsiella spp*. -producing ESBLs such as SHV and TEM types, have been established since the 1980s as a major cause of hospital-acquired infections. However, during the late 1990s, several community-acquired pathogens that commonly cause urinary tract infections and diarrhea have also been found to be ESBL producers (Paterson and Bonomo, 2005; Pitout *et al*., 2005). The CTX-M type of ESBLs are the most widespread enzymes, distributed both over wide geographic areas and among a wide range of clinical bacteria, in particular, members of the family of *Enterobacteriaceae*. They were initially reported in the second half of the 1980s, and their rate of dissemination among bacteria in most parts of the world has increased dramatically since 1995. At present, the CTX-M family comprises 67 enzymes. The phylogenic study reveals five major groups of acquired CTX-M enzymes (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9 and CTX-M-25) (Ben Achour *et al*., 2009).

Class B β-lactamases, termed metallo-β-lactamases, which are the most clinically important carbapenemases because they are capable of hydrolyzing all β-lactam antimicrobial agents, except the monobactam subgroup, and are not susceptible to β-lactamases inhibitors such as tazobactam, clavulacic acid and sulbactam (Walsh *et al.*, 2005). Currently, the carbapenem hydrolyzing β-lactamases (metallo β-lactamases) belong to the Bush and Jacoby group 3 classification of β-lactamases (Bush and

Jacoby, 2010). The global spread of acquired metallo-β-lactamases (MBLs) has raised serious concern because of their ability to confer resistance of Gram-negative bacteria to a broad spectrum of β-lactam antibiotics, including carbapenems. The infections, which are caused by such bacteria, are believed to result in high mortality as well as high healthcare costs and prolonged hospitalization. Therefore regular monitoring of the incidence of the β-lactamase producing organisms has become essential. To date, nine MBL types, namely, IMP-like, VIM-like, SPM-1, GIM-1, SIM-1, AIM-1, KHM-1, NDM-1 and DIM-1, have been identified in Gram negative bacilli (Hammami *et al*., 2011). The emergence of the most recently described carbapenemase, namely, the New Delhi metallo-β-lactamase (NDM-1), constitutes a critical medical issue. Indeed, this enzyme compromises the efficacy of almost all β-lactams (except aztreonam), including the last resort carbapenems. Although the spread of *bla*-$_{NDM}$-like genes (several variants) is derived mostly by conjugative plasmids in *Enterobacteriaceae*, this carbapenemase has also been reported from *Acinetobacter* spp. and rarely from *P. aeruginosa*. The Indian subcontinent, the Balkans regions, and the Middle East are considered the main reservoirs of NDM producers (Dortet *et al*., 2014). These are located within a variety of integron structures, where they have been incorporated as gene cassettes. When these integrons become associated with plasmids or transposons, transfer between bacteria is readily facilitated (Queenan and Bush, 2007). Five classes of integrons are known to play a role in the dissemination of antibiotic resistance, and the most extensively studied was class 1 integrons

(Mazel, 2006). MBL encoding genes are usually found as gene cassettes in class 1 integrons (Shibata *et al.*, 2003). Integrons are genetic elements encoding the components of a site specific recombination system that recognizes and captures mobile gene cassettes, mostly resistance determinants (Recchia and Hall, 1995). Such elements may be located within transposons, which in turn contribute actively to the dissemination of resistance determinants to aminoglycosides and β-lactams among Gram-negative species (Partridge *et al.*, 2002). In addition, it has been demonstrated that integrons and transposons are associated with the spread of resistance to third-generation cephalosporins when they encode extended-spectrum β-lactamases (ESBLs) (Luzzaro *et al.*, 2001).

Enterobacterial repetitive intergenic consensus (ERIC) PCR is a PCR-fingerprinting technique which is not arbitrary. The ERIC sequences are present in many copies in the genomes of different *Enterobacteriaceae*. ERIC elements are highly conserved at the nucleotide level, their positions in enterobacterial genomes varies between different species and has been used as a genetic marker to characterize isolates within a bacterial species. In ERIC-PCR a band pattern is obtained by amplification of genomic DNA located between successive repetitive ERIC elements or between ERIC elements and other repetitive DNA sequences for subtyping different Gram-negative enteric bacteria (Zulkifli *et al*., 2009).

1.2. Literature review

Klebsiella spp. are known to show resistance to penicillins, especially ampicillin and carbenicillin (Janda and Abbott, 2006). Since more and

more strains of *Klebsiella* spp. appear to be developing and harboring extended-spectrum beta-lactamases (ESBLs), cephalosporinases, and carbapenemases, resistance of *Klebsiella* spp. to current antibiotics appears to be increasing (Podschun and Ullmann, 1998; Janda and Abbott, 2006; Abbott, 2007). According to results from some studies in Europe and USA, ranges of susceptibility were as follows (Janda and Abbott, 2006): ceftriaxone (96-98%), ceftazidime (92-95%), gentamicin (95-96%), cefotaxime (96%), imipenem (98-100%), amikacin (98-99%), triethoprimsulfamethoxazole (SXT), (88-90%) piperacillin-tazobactam (90-97%). Resistance values tend to be higher for strains isolated from ICU patients compared to non-ICU patients. Pan-resistant isolates have been identified in India. In new study from Pakistan, the ESBL producing *Klebsiella* spp., showed maximum resistance to ceftazidime (100%), cefotaxime (89%) and cefuroxime (84%) while minimum resistance was seen with imipenem (4%), nitrofurantoin and piperacillin/tazobactam (8%) (Majda *et al*., 2013).

ESBLs have emerged worldwide as a significant cause of community and health care associated infections. In a 1997-1998 survey of 433 isolates from 24 intensive care units in western and southern Europe, 25% of *Klebsiella* spp., possessed ESBLs (Babini and Livermore, 2000). Another large study from more than 100 European intensive care units found that the prevalence of ESBLs in *Klebsiella* ranged from as low as 3% in Sweden to as high as 34% in Portugal (Hanberger *et al*., 1999). A third study, which has been done in 2001 in two Greek hospitals, revealed that

58% of *K. pneumonia* isolates were characterized as ESBL producers, and showed that SHV-5-type ESBLs were the most frequent followed by CTX-M (Tzelepi *et al*., 2003). In Turkey, a survey of *Klebsiella* spp. from intensive care units from eight hospitals showed that 58% of 193 isolate harbored ESBLs (Gunseren *et al*., 1999). First reports of ESBL-producing organisms in the United States occurred in 1988 (Jacoby and Medeiros, 1991). In Latin America, previous data suggested that many centers in different countries *K. pneumonia* have about 45% phenotypes consistent with ESBL production (Winokur *et al*., 2001); whereas in the United States only 3.3% of *E. coli* and 8% of *K. pneumonia* have such phenotype (Neuhauser *et al*., 2003). In South America, ESBLs have been found in 30 to 60% of *Klebsiella* spp., isolated from intensive care units in Brazil, Venezuela and Colombia (Pfaller *et al*., 1993; Sader *et al*., 2000; Otman *et al*., 2002).

Recently, ESBL screen test was positive in 96% of *K. pneumoniae* isolates positive. It was reported that AmpC production was 12.5% more compared to ESBL and MBL production (8% both), and the occurrence of co-production of AmpC and MBL was found in 67% of *K. pneumoniae* isolates recovered from blood samples of suspected cases of neonatal septicemia (Gajul *et al*., 2015). In another study, it was shown that 37.5% and 33.3% of *Klebsiella* spp. were ESBL and MBL producers, respectively (Wadekar *et al*., 2013).

In a study carried out by Vinod Kumar *et al*. in India, it was reported that the rate of MBL producers among *K. pneumoniae* isolates were 27%

(Vinod Kumar *et al*., 2013). *Klebsiella pneumoniae* isolates collected in the Czech Republic from 16 hospitals, 24.2% were determined to be ESBL-positive. The frequency of ESBL positive isolates was significantly higher in intensive care units (39.9%) than in other hospital wards (13.1%). More than 50% of ESBL-positive isolates were treated effectively with only meropenem (98%), cefoperazone/sulbactam (61%) and amikacin (54%) (Kolar *et al*., 2006). It was reported that 89% *K. pneumoniae* isolates were positive in ESBL screening (Modi *et al*., 2012). Oberoi *et al*., reported that the prevalence of β-lactamases in North India was 79.6% among *K. pneumoniae* isolated from ICU patients. Distribution of various β-lactamases in these clinical isolates was 34.1%, 22.7%, 2.3%, 9.1%, 2.3%, 9.1% for ESBL, MBL, AmpC, ESBL/MBL, AmpC/MBL and ESBL/AmpC, respectively (Oberoi *et al*., 2013). Altun *et al*., reported that the prevalence of ESBL, AmpC among *K. pneumoniae* obtained mainly from ICU patients was 73%, 47%, respectively (Altun *et al*., 2013). A study from Kuwait showed high prevalence of VIM-4 and NDM-1 metallo-β-lactamase among carbapenem-resistant *Enterobacteriaceae* including *Klebsiella* spp. It was shown that *K. pneumoniae* isolates produced the MBL NDM-1 and co-produced the plasmid-encoded AmpC CMY-4. The VIM-4-producing isolates co-produced extended-spectrum B-lactamases including CTX-M-15 and some SHV derivatives (Jamal *et al*., 2013). In Iraq it was reported that ESBL production among *K. pneumoniae* isolates was 80% (Jarjees, 2006). In United Arab Emirates, who found that the ESBL production in *K. pneumoniae* was 42% (Al-Zarouni *et al.*, 2008). In

Egypt during 2004-2005 it was reported that 61.5% *K. pneumoniae* isolates were ESBL producers (Fam *et al*.,2006). In the same period it was 24.3% in *K. pneumonia* in Bahrain (Bindayna *et al*., 2009) ; whereas it was 77.7% in *K. pneumonia* in (2005-2007) in Iran (Mehrgan *et al*., 2010). In Saudi Arabia in 2007, phenotypic characterization identified a high ESBL rate of 55% of *K. pneumonia* isolates, the prevalence of *SHV*, *TEM* and *CTX-M* β-lactamase genes were 97.3%, 84.1% and 34.1%, respectively. Within the CTX-M family, two groups of enzymes, CTX-M-1 and CTX-M-9-like genes were found with prevalence of 60% and 40%, respectively (Al-Agamy *et al*., 2009). In Jordan in 2004, 71.4% were ESBL producers in *K. pneumonia* and 28.6% in *K. oxytoca* (Batchoun *et al*., 2009). A study in Algeria in 2009, reported that the prevalence of ESBL producers in *K. pneumonia* was 17.4%, CTX-M-1 is predominant (Nedjai *et al*., 2012). In France, 10 to 30 % of *K. pneumoniae* strains are reported to produce plasmid mediated ESBLs of the TEM or SHV families (Jacoby and Medeinos, 1991).

In Poland, in three pediatric hospitals, 58.2% of *K. pneumoniae* strains with multiple ESBLs were found, including five non-clonal strains (4.5%) harboring bla genes for ESBLs of three families (CTX-M, SHV and TEM). *K. pneumoniae* strains harboring bla genes for TEM-130 and TEM-132 ESBLs were also detected (Dzierzanowska *et al*., 2010). In Japan it was found that 65.4% in *K. pneumoniae* isolates were ESBL enzymes producers. Bareja *et al*., (2013), reported that 28.7% and 13.4% of *Klebsiella* spp. were ESBL and AmpC positive, respectively. Also it was

reported that 71%, 9.7% and 9.7% of *K. pneumoniae* recovered from suspected cases of neonatal sepsis were ESBL, AmpC and co-existence of ESBL and AmpC producers, respectively. However, 75% of *K. oxytoca* were ESBL producers. TEM gene occurred 31.8% of *K. pneumoniae* and 33.3% of *K. oxytoca* were ESBL producers (Chelliah *et al*., 2014). In Iran, it was reported that 44% of *K. pneumoniae* isolates were ESBLs producers, 28% produced AmpC β-lactamases and 1.3% produced MBLs. Simultaneous production of ESBLs and AmpC β-lactamases were also observed in 28% isolates of *K. pneumoniae* (Mansouri *et al*., 2014). Bhattacharjee *et al*., (2008) recorded that the prevalence ESBL producing *K. pneumonia* was 62.7% among *K. pneumonia* isolates; whereas Jain *et al*., (2003) showed that ESBL production was reported in 86.6% of *K. pneumoniae*. As per Gandhi *et al*., (2013), ESBL production was seen in 50% of *K. pneumoniae* isolates. In New Zealand, the prevalence of ESBLs was estimated to be 4.2% among *Klebsiella* spp. Clearly, CTX-M ESBLs, in particular CTX-M-15, are almost wholly dominant among ESBL-producing *K. pneumoniae*. Other types CTX-M-14, CTX-M-68 and SHV-2a were also reported (Heffernan *et al*., 2009). In Tunisia, the prevalence of ESBL-producing *K. pneumoniae* strains was ranged from 32.4%-37.5% (Messai *et al*., 2007; Abbassi *et al*., 2008). In India, it was reported that 67% of *Klebsiella* spp were β-lactamases NDM-1 producing strains (Bhaskar *et al*., 2013). NDM-1 have now been found in multiple areas of India and Pakistan and in the United Kingdom (Kumarasamy *et al*., 2010). Recently in Nepal, it was reported that 21.08% *K. pneumoniae* strains were

suspected to be carbapenemase-producers and 71.79% of *K. pneumoniae* isolates producing MBL were found to be “pandrug- resistant (Bora *et al*., 2014); whereas in Logos, Nigeria the prevalence of MBL during 2008 was 53% in *K. pneumoniae* (Enwuru *et al*., 2011). In India, Among the ESBL and MBL genes, CTX-M was detected in 37.33 %, SHV was detected in 45.33 % and TEM was detected in 47.33 %, NDM-1 was detected in 6.25 %, and KPC and VIM were detected in 7.9 % and 17.3 % *K. pneumoniae* isolates, respectively. However, the *blaSHV* gene was found to be associated with the *blaTEM* in 32.0 % of isolates. (Chaudhary and Payasi, 2013). The proportion of imipenem-resistant *K. pneumoniae* has increased from less than 1% in 2001, to 20% in isolates from hospital wards and to 50% in isolates from ICUs in 2006 (Vatopoulos, 2008).

In Taiwan, CTX-M and SHV-type ESBLs with CMY- and DHA type AmpC enzymes are the most common β-lactamases that conferred resistance to extended-spectrum cephalosporins in clinical *K. pneumoniae* isolates (Yan *et al*., 2004, Yan *et al*., 2006). The emergence of a multidrug resistant *K. pneumoniae* isolates, which produce VIM-4, CTX-M-15, TEM-1, CMY-4 have been reported from France (Ktari *et al*., 2006). Co-existence of CMY-8 and CTX-M-3 was reported in a 269 kb conjugative plasmid from *K. pneumoniae* (Chen *et al*., 2007). A recent report for co-existence of CMY-6 and CTX-M-15 has recently been reported on the similar type of plasmid from India (Shahid *et al*., 2009). In Tunisia, CTX-M and SHV enzymes have been reported in *K. pneumoniae* and *E. coli* strains isolated from humans or food samples of animal origin (Mamlouk *et*

al., 2006; Ktari *et al*., 2006; Jouini *et al*., 2007; Abbassi *et al*., 2008; Ben Achour *et al*., 2009).

Among the MBLs detected in *K. pneumoniae* IMP-1 and IMP-8 have been described from Singapore, Japan and Taiwan (Senda *et al*., 1996; Podschun and Ullmann, 1998; Koh *et al*., 1999). IMP-1 has been described from Brazil (Lincopan *et al*., 2006), Turkey (Aktas *et al*., 2006), and UK (Woodford *et al*., 2007). VIM-1 and VIM-4 have been described in Greece and Italy (Giakkoupi *et al*., 2003; Luzzaro *et al*., 2004). In 2008 in Lebanon, CTX-M-15 has been described and the first isolation MBL producing *K. pneumonia* (blaIMP-1) has been detected also (Daoud *et al*., 2008). The first isolation MBL (IMP-1) producing *K. pneumoniae* in Brazil was in 2003 (Lincopan *et al*., 2006). CTX-M-28 has been described from Tunisia (Ben Achour *et al*., 2009). Recently, NDM-1-producing *K. pneumoniae* has been detected from Vietnam (Hoang *et al*., 2013).

The first report of NDM-1 producing *K. pneumoniae* in Iran was in 2013 (Shahcheraghi *et al*., 2013); whereas it was reported in Slovakia in 2014 (Lovayová *et al*., 2014). In northern Italy, an Outbreak of NDM-1-producing *Enterobacteriaceae* including *K. pneumoniae* was reported in 2011 (Gaibani *et al*., 2011). Another report from United Arab Emirates in 2009–2011 revealed the emergence and spread of NDM-1 producer *K. pneumoniae* (Sonnevend *et al*., 2013). In Ireland, first *K. pneumoniae* carbapenemase (KPC)-producers were in 2009 (Roche *et al*., 2009).

1.3 Aims of the study:

Little information is available about the prevalence and molecular characterization of β-lactamases (ESBLs, MBLs and AmpC β-lactamases) in Palestine. The Specific aims of the present study were:

(1) to determine the molecular epidemiology of MBL, ESBL and AmpC β-lactamase producing *K. pneumoniae* isolates using molecular techniques;

(2) to determine the susceptibility pattern to different classes of antibiotics

(3) to assess the prevalence of class 1, 2 3 and 4 integrons in clinical *K. pneumoniae* isolates.

Chapter Two

Materials and Methods

2.1. Sample collection and *Klebsiella pneumoniae* identification:

A total of 51 isolates of *Klebsiella pneumoniae* (11 blood, 18 urine, 5 sputum, 17 swab) were isolated from inpatients and outpatients at An-Najah Natinal University Hospital- Nablus (n=16), Rafidia Hospital-Nablus (n=2) and al-Watani (the National) Hospital –Nablus (n=2), The Martyar .Dr. Khalil. S. Hospital-Jenin (n=8), Al-Amal Hosptal-Jenin (n=1), AL-Razi Hospital- Jenin (n=1), Al-Shamal Lab- Jenin (n=7), Thabat hospital-Tulkarem (n=14) during September-December 2015. The isolates were identified by API 20 E system in laboratories of these hospitals. In addition, isolates were confirmed in Microbiology laboratories at An-Najah National University-Nablus, Palesine; using different tests such as growth on MacConkey, detection of growth at 5°C and 44.5°C, Gram stain reaction, motility test and other biochemical tests were used such as Indole test and Voges-Proskauer test (Brisse *et al*., 2006).

2.2. Media preparation

2.2.1. MacConkey Agar

MacConkey agar (HIMEDIA, India) was prepared according to the manufacturer's instructions. A 2L bottle containing 1000 ml of deionized water and 50 g of the medium was heated and stirred until completely dissolved. The solution was allowed to boil for 1min, and then autoclaved at 121°C for 15 min. After that it was allowed to cool to approximately 50-60°C, the melted agar was poured into Petri dishes to have about 20-25 ml

in each, then covered and left overnight at room temperature. On the next day, Petri dishes were stored in refrigerator at 4°C.

2.2.2. Sulfide Indole Motility (SIM) medium

SIM medium (Acumedia, USA) was prepared according to the manufacturer's instructions. A 1L bottle containing 500 ml of deionized water and 15 g of the medium was heated and stirred until completely dissolved. A total of 10 ml of medium was distributed into each tube and plugged with cotton. Then the medium was autoclaved at 121°C for 15 min, allowed to cool and then stored in refrigerator at 4°C.

2.2.3. Mueller Hinton Agar (MHA)

Mueller Hinton agar (BD, Baltimore) was prepared according to manufacturer's instructions. In a 1 L bottle, 500 ml of deionized water were mixed with 19 g of the medium, heated and stirred until completely dissolved. The solution was allowed to boil for 1 minute, and then autoclaved at 121 °C for 15 minutes. After that it was allowed to cool to approximately 50-60°C, the melted agar was poured into sterile Petri dishes to have approximately 25 ml each, then covered and left overnight at room temperature. On the next day, Petri dishes were stored in refrigerator at 4°C.

2.2.4. Nutrient Agar (NA)

Nutrient agar (ACUMEDIA, USA) was prepared according to manufacturer's instructions. In a 2 L bottle, 1L of deionized water was mixed and heated with 11.5 g of the medium until the agar completely

dissolved. The solution was allowed to boil for 1 minute, and then autoclaved at 121°C for 15 minutes. After that it was allowed to cool to about 50-60°C, the melted agar was poured into sterile Petri dishes to have approximately 20-25 ml each then covered and left overnight at room temperature. On the next day, Petri dishes were stored in refrigerator at 4°C.

2.2.5 Tryptone Soy Broth (TSB)

Tryptone Soy broth (OXOID, England) was prepared according to manufacturer's instructions labeled on the bottle. In a 0.5 L bottle, 250 ml of deionized water were mixed and boiled with 7.5 g medium until completely dissolved. The broth was then distributed into tubes to have approximately 10 ml each and plugged with cotton. Then tubes were autoclaved at 121 °C for 15 minutes, allowed to cool and then stored in refrigerator at 4°C.

2.2.6. Methyl Red-Voges Proskauer (MR-VP)

MR-VP medium (HIMEDIA, India) was prepared according to the manufacturer's instructions. A 0.5 L bottle containing 250 ml of deionized water and 4.3 g of medium were mixed thoroughly, heated until completely dissolved the components. The broth was then distributed into tubes to have approximately 5-8 ml each and plugged with cotton. Then tubes were autoclaved at 121°C for 15 min, allowed to cool and then stored in refrigerator at 4°C.

2.3. Bacterial identification

2.3.1. Gram staining

Prepared bacterial smear from Tryptone Soy broth was fixed by heating, then fixed smear was stained as previously described (Cappuccino and Sherman, 1996).

2.3.2. Motility test

A motile organism, caused turbidity (diffused growth) in the SIM agar deep tube inoculated by means of stab method and the stab line is obscured. But in case of a nonmotile organism, the growth was restricted to the stab line and the medium was very clear (Johnson and Case, 1998).

2.3.3. Indole test

SIM agar deep tube inoculated with a pure culture of the *K. pneumoniae* by mean of stab method for motility and incubated for 24 hours at 37ºC Indole test was carried out by adding 0.5 ml of Kovac's reagent as previously described(Cappuccino and Sherman, 1996). Positive result, a cherry-red ring (pink to red color) appeared on the top of medium after adding the indicator, while negative result showed no color change after the addition of the indicator.

2.3.4. VP test

MR/VP broth inoculated with a pure culture of the *K. pneumoniae* and incubated for 24 hours at 37ºC. Test was carried out by adding 0.5 ml of Barritt's reagent A, the culture was shaken, then immediately another 0.5

ml of Barritt's reagent B was added, then culture was shaken and re-shaken after every 3-4 min. Formation a cherry red color after 15 min indicates a positive result for VP test while a yellow-brown or no change in color indicates a negative result (Cappuccino and Sherman 1996).

2.3.5. Growth at 5°C and 44.5°C

Each *K. pneumoniae* isolate was subcultured on 2 MacConkey agar plates, one was incubated at 5°C, while the other plate was at 44.5°C for 24 h.

2.4. Susceptibility test

Susceptibility of bacterial isolates to antibiotics was determined according to the Clinical and Laboratory Standard Institute (CLSI) using the disk diffusion method (CLSI, 2012). Priefly all *K. pneumoniae* isolates were tested using disks (Oxoid, England) to determine resistance against Ceftazidime (CAZ, 30µg), Cefotaxime (CTX, 30µg), Imipenem (IPM, 10µg), Meropenem (MEM, 10µg), Ceftriaxone (CRO, 30µg) and Aztreonam (ATM, 30µg). The MHA plates were swabbed from pure bacterial broth, disks were then added and lightly pressed on agar surface Then the plates were incubated at 37°C for 18-24 hrs. Inhibition zones were measured in millimeters. Isolates were classified as sensitive, intermediate or resistant according to the criteria recommended by CLSI guidelines (CLSI, 2012).

2.5. DNA extraction

K. pneumoniae genome was prepared for PCR according to the method described previously (Adwan *et al*., 2013). Briefly, cells from overnight

nutrient agar plate culture, washed with 1 ml of 1X Tris-EDTA buffer (10 mM Tris-HCl, 1 mM EDTA [pH 8]), then the pellet was resuspended in 0.4 ml of sterile distilled H_2O, boiled for 10-15 min, then cells were incubated on ice for 10 min. By centrifugation at 11,500 X *g* for 5 min, the debris was pelleted. Supernatant was transferred into new tube and DNA concentration was determined using a spectrophotometer, then DNAsamples were stored at -20ºC until use for further analysis.

2.6. Multiplex PCR amplification

2.6.1. Detection of MBL genes

Detection of gene encoding for the *VIM*, *IMP*, *SPM-1*, *GIM-1* and *SIM-1* enzymes was performed by multiplex PCR according to method previously described (Ellington *et al.*, 2007). Sequences of primers and size of amplicons are described in Table 2.1. Briefly, PCR reactions were carried out in a total volume of 50 µl of the mixture containing 25 µl of PCR premix with MgCl2 (Sigma), 0.4 µM of each primer, 5 µl of genome template. PCR was performed using a thermal cycler (Mastercycler Personal, Eppendorf) under the following conditions: initial denaturation at 94°C for 5 min; was followed by 36 cycles by denaturation at 94°C for 30 sec, annealing at 52°C for 40 sec and extension at 72°C for 50 sec; with a final extension at 72°C for 5 min. Amplified PCR products were detected using a 1.5 % agarose gel stained with ethidium bromide.

2.6.2. Detection of *KPC, NDM* and *DIM* genes

Detection of gene encoding for the *KPC, NDM* and *DIM* enzymes was performed by multiplex PCR according to method previously described (Poirel *et al*., 2011). Sequences of primers and size of amplicons are described in Table 2.1. PCR reactions and conditions were used for amplification as well as described in section 2.6.1.

2.6.3. Detection of AmpC β-lactamase genes

Amplification of plasmid mediated AmpC β-lactamase (*ampC*) genes in all *K. pneumoniae* isolates were carried out by PCR as previously described (Pérez-Pérez and Hanson, 2002). Primer nucleotide sequences and the expected size of amplicons for genes are presented in Table 2.1. PCR reactions were performed as described in section 2.6.1. Amplification of genes was performed under the following conditions: initial denaturation at 94°C for 3 min; was followed by 25 cycles of denaturation at 94°C for 30 sec, annealing at 64°C for 30 sec and extension at 72°C for 1 min; with a final extension at 72°C for 5 min.

2.6.4. Detection of ESBL (*TEM, SHV, CTX-M* and *OXA*) genes

Amplification of gene sequences coding for the TEM, SHV, CTX-M and OXA enzymes was performed by the multiplex PCR. The oligonucleotide primer sets and the expected size of amplicons (bp) for genes are presented in Table 2.1. PCR reactions were performed as described in section 2.6.1. Amplification of genes was performed under the following conditions: initial denaturation at 94°C for 5 min; was followed by 25 cycles of

denaturation at 94°C for 30 sec, annealing at 60°C for 30 sec and extension at 72°C for 2 min; with a final extension at 72°C for 5 min.

2.6.5. Detection of class 1, 2, 3 and 4 integrons

All *K. pneumoniae* isolates were screened for the presence of integrase genes *intI1*, *intI2*, *intI3* and *intI4* using primers previously described (Shibata *et al.*, 2003). Sequences of primers and size of amplicons are described in Table 2.1. PCR reactions were performed as described in section 2.6.1. The amplification was carried out according to the following thermal conditions: denaturation at 94ºC for 30 sec, annealing at 58ºC for 30 sec, and extension at 72ºC for 1 min for 30 cycles, with a final extension step at 72ºC for 2 min

2.6.6. ERIC-PCR

ERIC-PCR was carried out 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. Briefly, PCR reactions were carried out in a total volume of 50 µl of the mixture containing 25 µl of PCR premix with MgCl2 (Sigma), 1µM of each primer, 5 µl DNA template. In addition, the master mix was modified by increasing the concentration of dNTPs to 400µM , 3mM MgCl2 and 2.5 U of Taq DNA polymerase for each reaction. Amplification of DNA was carried out according to the following thermal conditions: initial denaturation for 3 min at 94ºC was followed by 40 cycles of denaturation at 94ºC for 50 sec, annealing at 50ºC for 40 sec and extension at 72ºC for 1 min, with a final extension step at 72ºC for 3 min.

The amplified products were analyzed by electrophoresis on 1.5% agarose gel. Images of gel were scored using binary scoring system that recorded 0 and 1 for absence and presence of bands, respectively. A binary matrix was analyzed by the unweighted pair group method for arithmetic averages (UPGMA), using SPSS statistical software version 20 (IBM).

2.7. Sequence homology and phylogenetic analysis

Amplified PCR products of some β-lactamases genes were purified by the MinElute PCR purification kit (Qiagen, Hilden, Germany) and sequenced by dideoxy chain termination method using ABI PRISM sequencer, model 3130 (Hitachi Ltd, Tokyo, Japan), at Bethlehem University, Bethlehem, Palestine. Sequence information for these genes were further submitted to Genbank for accession numbers.

The comparison of the sequences of this research was made with available sequences for β-lactamases genes previously deposited in Genbank using BLAST system. Multiple sequence alignment was carried out using ClustalW of MEGA software (version 5) and the evolutionary distances were computed using the Maximum Composite Likelihood method. Phylogenetic relationships were based on alignments obtained from ClustalW of a 264 bp sequence. Neighbor-Joining program in the same software was used to construct the phylogenetic tree. The robustness of the groupings in the Neighbor Joining analysis was assessed with 1000 bootstrap resamplings.

Table 2.1. Beta-lactamases target genes and ERIC sequences for PCR amplification, amplicon size and primer sequences

Group	Targets	Primer sequence 5′→3′	Expected amplicon size (bp)	Primer mix	References
AmpC β-lactamase (Class C)	*MOX-1, MOX-2, CMY-1, CMY-8 to CMY-11*	MOXM F GCT GCT CAA GGA GCA CAG GAT MOXM R CAC ATT GAC ATA GGT GTG GTG C	520	1	Pérez-Pérez and Hanson, 2002
	LAT-1 to LAT-4, CMY-2 to CMY-7, BIL-1	CITM F TGG CCA GAA CTG ACA GGC AAA CITM R TTT CTC CTG AAC GTG GCT GGC	462	1	Pérez-Pérez and Hanson, 2002
	DHA-1, DHA-2	DHAM F AAC TTT CAC AGG TGT GCT GGG T DHAM R CCG TAC GCA TAC TGG CTT TGC	405	1	Pérez-Pérez and Hanson, 2002
	ACC	ACCM F AAC AGC CTC AGC AGC CGG TTA ACCM R TTC GCC GCA ATC ATC CCT AGC	346	1	Pérez-Pérez and Hanson, 2002
	MIR-1T, ACT-1	EBCM F TCG GTA AAG CCG ATG TTG CGG EBCM R CTT CCA CTG CGG CTG CCA GTT	302	1	Pérez-Pérez and Hanson, 2002
	FOX-1 to FOX-5b	FOXM F AAC ATG GGG TAT CAG GGA GAT G FOXM R CAA AGC GCG TAA CCG GAT TGG	190	1	Pérez-Pérez and Hanson, 2002
extended spectrum β-lactamases	*OXA*	OXA F 5-ATT ATC TAC AGC AGC GCC AGT G-3 OXA R 5-TGC ATC CAC GTC TTT GGT G-3	296	2	Kim *et al*., 2009

(Class D)					
extended spectrum β-lactamases (Class A)	*SHV*	SHV F 5-ATG CGT TATATT CGC CTG TG-3 SHV R 5-TGC TTT GTT ATT CGG GCC AA-3	747	2	Paterson *et al*., 2003
	TEM	TEM F 5-TCG CCG CAT ACA CTA TTC TCA GAA TGA-3 TEM R5- ACG CTC ACC GGC TCC AGA TTT AT-3	445	2	Monstein *et al*., 2007
	CTX	CTX-M F ATG TGC AGY ACC AGT AAR GTK ATG GC CTX-M R TGG GTR AAR TAR GTS ACC AGA AYC AGC GG	593	2	Boyd *et al*., 2004
	KPC	KPCM F CGTCTAGTTCTGCTGTCTTG KPCM R CTTGTCATCCTTGTTAGGCG	789	3	Poirel *et al*., 2011
Metallo-β-lactamases	*NDM*	NDM F 5-GGTTTGGCGATCTGGTTTTC -3 NDM R 5-CGGAATGGCTCATCACGATC -3	621	3	Poirel *et al*., 2011
	DIM	DIM F 5- GCTTGTCTTCGCTTGCTAACG-3 DIM R 5- CGTTCGGCTGGATTGATTTG-3	699	3	Poirel *et al*., 2011
Metallo-β-lactamases (Class B)	*Imp*	Imp-F 5-GGAATAGAGTGGCTTAAYTCTC-3 Imp-R 5-CCAAACYACTASGTTATCT-3	188bp	4	Ellington *et al*., 2007
	Vim	Vim-F 5-GATGGTGTTTGGTCGCATA-3 Vim-R 5-CGAATGCGCAGCACCAG-3	390bp	4	Ellington *et al*., 2007
	Gim	Gim-F 5-TCGACACACCTTGGTCTGAA-3 Gim-R 5-AACTTCCAACTTTGCCATGC-3	477bp	4	Ellington *et al*., 2007
	Spm	Spm-F 5-AAAATCTGGGTACGCAAACG-3 Spm-R 5-ACATTATCCGCTGGAACAGG-3	271bp	4	Ellington *et al*., 2007
	Sim	Sim-F 5-TACAAGGGATTCGGCATCG-3 Sim-R 5-TAATGGCCTGTTCCCATGTG-3	570bp	4	Ellington *et al*., 2007

Integrases	*intI1*	intI1 F 5-GCATCCTCGGTTTTCTGG-3 intI1 R 5-GGTGTGGCGGGCTTCGTG-3	457bp	5	Shibata *et al*., 2003
	intI2	intI2 F 5-CACGGATATGCGACAAAAAGG T-3 intI2 R 5-GTAGCAAACGAGTGACGAAATG-3	789bp	5	Shibata *et al*., 2003
	intI3	intI3 F 5-AT TGCCAAACCTGACTG-3 intI3 R 5-CGAATGCCCCAACAACTC-3	922bp	5	Shibata *et al*., 2003
	Intl4	Intl4 F: 5-CGGTATGTCTAATTGCTCTTG-3 Intl4 R: 5- TGGCCACAAAGACTCAATCAC-3	696bp	5	Goldstein *et al*., 2001

Chapter Three

Results

3.1. Identification of *K. pneumoniae* isolates

A total of 51 of *K. pneumoniae* isolates were identified in different laboratories of hospitals using API 20 E system. In addition, these isolates were confirmed in Microbiology laboratories at An-Najah National University-Nablus, Palestine. On MacConkey agar, all these clinical isolates formed typical rose pink mucoid colonies, lactose fermentation positive, while on nutrient agar they showed mucoid, circular, convex small colonies. Bacterial cells of these isolates showed Gram-negative reaction and bacilli shape In addition, all isolates showed indole and H_2S negative, VP test positive, and were non motile typical. All of these isolates showed a growth on MacConkey agar plates at 44.5◦C but not at 5◦C.

3.2. Antibiotic susceptiblity

Results of the current study showed that all *K. pneumoniae* isolates were susceptible to Imepenem, while 68.6% and 54.9% of the isolates were resistant to Meropenem and Cefotaxime, respectively. The resistance pattern of these isolates against six chosen antimicrobial agents is presented in Table 3.1.

Table 3.1. Antibiotic resistance of 51 *K. pneumoniae* isolates recovered from different clinical samples.

Antibiotic	Resistant strains	
	No.	%
Cetriaxone	27	52.9
Cefotaxime	28	54.9
Imipenem	0	0
Ceftazidime	21	41.2
Aztreonam	23	45.1
Meropenem	35	68.6

3.3. Detection of β-lactamases and integrons

The prevalence of possible ESBLs, MBLs and AmpC β-lactamase genes among the 51 studied clinical strains of *K. pneumoniae*, using multiplex PCR technique was (92.2%), (9.8) and (3.9%), respectively. The majority of possible ESBL producing *K. pneumoniae* isolates that had a single possible ESBL enzyme, were harboring the *TEM* gene with a prevalence of 43.1% and other isolates were carrying *SHV* and OXA genes with a prevalence of (17.6%) and (1.96%), respectively. Fifteen isolates were found to carry both *TEM* and *OXA* with the prevalence of (29.4%). The most common detected gene among the studied clinical isolates of *K. pneumoniae* was *TEM* gene with a prevalence of (72.5%). For MBLs and AmpC β-lactamases *NDM* and *DHA* genes were detected, respectively, among the collected isolates. In addition, six isolates (11.76%) showed coexistence with at least another type of β-lactamases. Prevalence of β-lactamases of this research are presented in Table 3.2 and Figure 3.1. Molecular analysis and Phylogenetic relationships showed that all

sequenced *TEM*, *SHV*, *OXA*, *NDM* and *DHA* genes belonged to *TEM-1*, *SHV-1*, *OXA-1*, *NDM-1* and *DHA-1*, respectively. Phylogenetic analysis of sequenced β-lactamases genes is presented in Figure 3.2. The nucleotide sequences reported in this study were deposited at the GenBank database under the accession numbers KU594303- KU594326.

A total of 26 of *K. pneumoniae* isolates (50.98%) harbored class 1 integrons, whereas other classes were not detected. Results are presented in Figure 3.3. All integrons were detected in *K. pneumoniae* isolates carrying β-lactamase genes.

Table 3.2. Prevalence of β-lactamase among 51 clinical isolates of *K. pneumoniae* detected by multiplex PCR technique.

β-Lactamases														
Extended spectrum β-lactamases No. (%)					AmpC β-lactamase No. (%)					Metallo-B-lactamases No. (%)				
Class (A)				Class (D)	Class (C)					Class (B)				
SHV	TEM	CTX-M	TEM and OXA	OXA	MOX, CMY, BIL	DHA	ACC	MIR-1T, ACT-1	FOX	Imp	Vim	Gim	Spm	NDM
9 (17.6)	22 (43.1)	0 (0)	15 (29.4)	1 (1.96)	0 (0)	2 (3.9)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5(9.8)
Total 47 (92.2)					Total 2 (3.92)					Total 5 (9.8)				

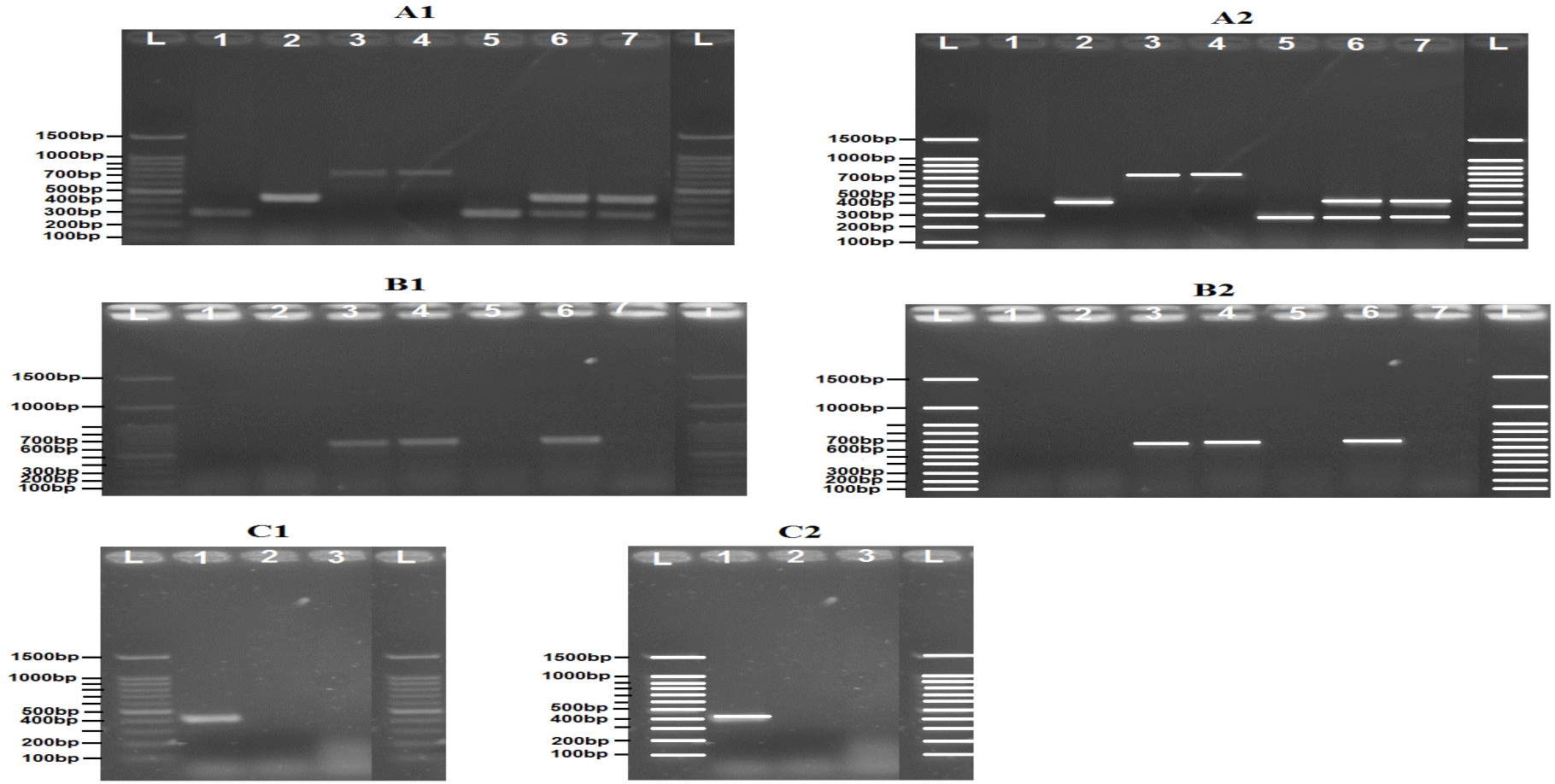


Figure 3.1. Multiplex PCR profiles specific for possible ESβLs, MBLs and/or AmpC β-lactamase genes detected in clinical isolates of *K. pneumoniae*.

L represented the ladder.

Figure A1 or A2 for ESβLs: Lanes 1 and 5 for *OXA* gene; Lane 2 for *TEM* gene; lanes 3 and 4 for *SHV* gene and Lanes 6 and 7 for *TEM* and *OXA* genes. Figure B1 or B2 for MBLs: Lanes 3, 4 and 6 for *NDM* genes. Figure C1 or C2 for AmpC β-lactamases: Lane 1 for *DHA* gene.

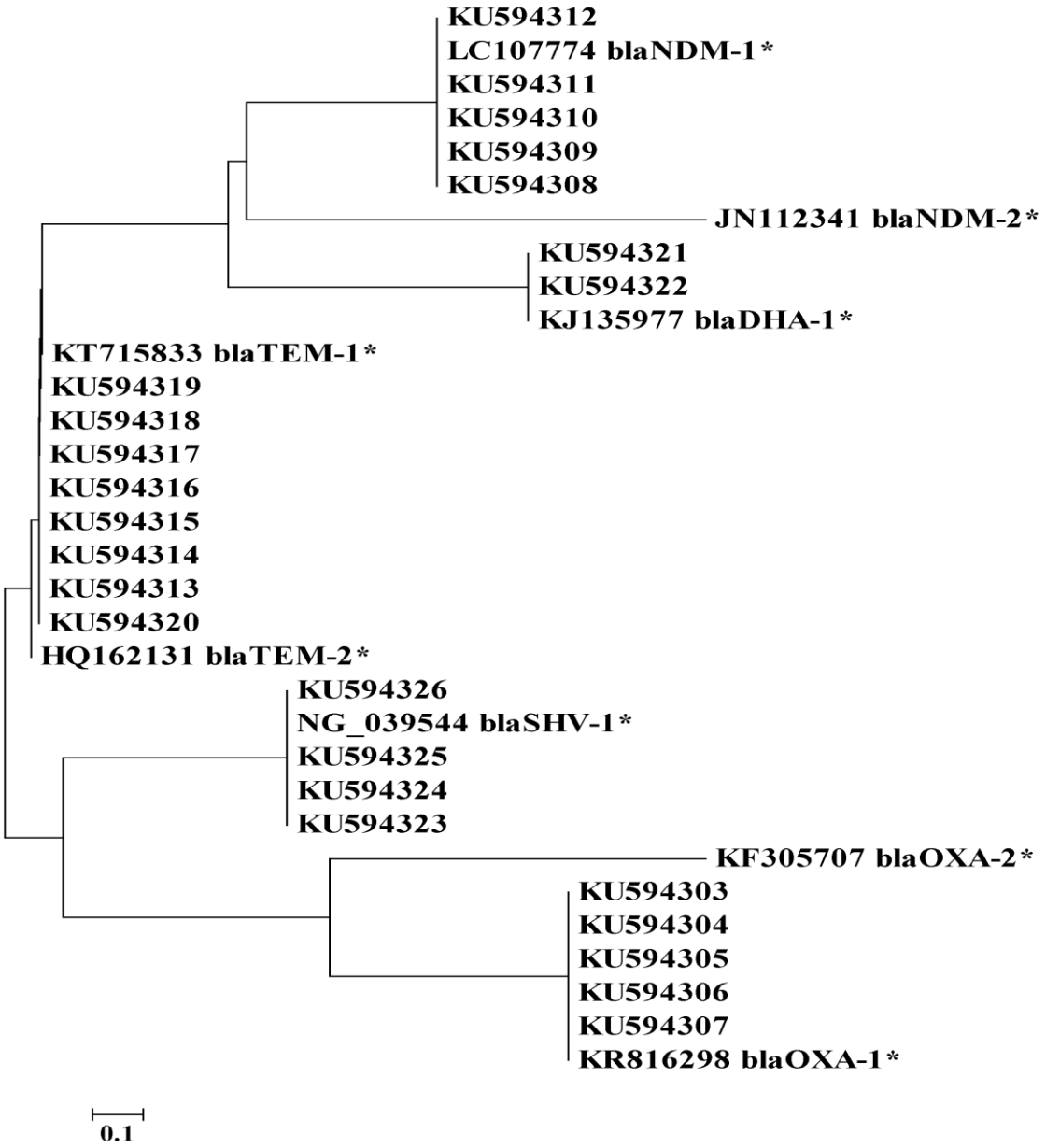


Figure 3.2. Phylogenetic analysis constructed by Neighbor-Joining method based on the partial *NDM-1*,*DHA-1*, *TEM-1*, *SHV-1* and *OXA-1* and gene nucleotide sequences.

Reference nucleotide sequences for the *NDM-1*, *NDM-2*, *DHA-1*, *TEM-1*, *TEM-2*, *SHV-1*, *OXA-1 and OXA-2* genes were denoted by asterisk and retrieved from genbank. The phylogenetic tree was bootstrapped with 1000 replicate, and the genetic distance corresponding is shown by the bar. The evolutionary distances were computed using the Maximum Composite Likelihood method. All positions containing gaps and missing data were eliminated. There were a total of 264 positions in the final dataset. Evolutionary analyses were conducted using MEGA version 5.

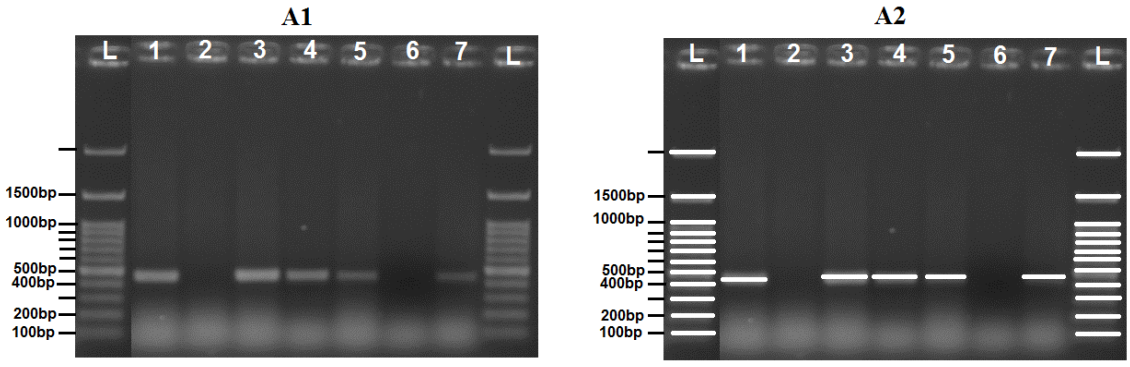


Figure 3.3. Multiplex PCR profile specific for integrons. L represented the ladder, Lanes 1, 3, 4 and 5 represented class I integron., Lane 6 represented negative control.

3.4. ERIC-PCR analysis

ERIC-PCR typing of 40 clinical isolates of *K. pneumoniae* harbored different β-lactamase genes were genetically diverse and consisted of a heterogeneous population with a total of 16 ERIC PCR profiles (clusters) at a 50% similarity level. Results of ERIC-PCR fingerprint are presented in Figures 3.4 and 3.5.

.

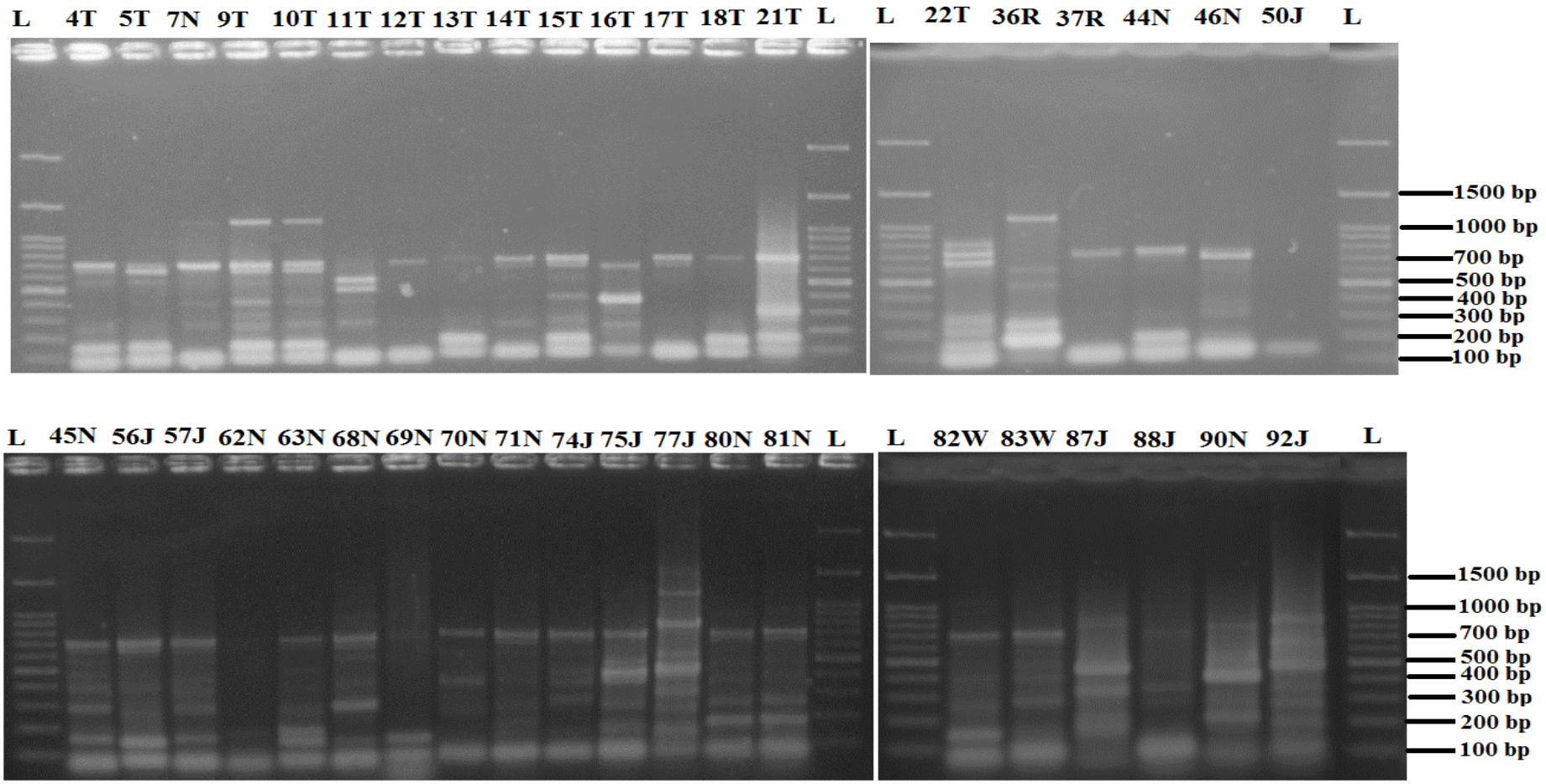


Figure 3.4. ERIC PCR profiles of 40 clinical *K. pneumoniae* isolates carried genes for ESβLs, MBLs and/or AmpC β-lactamases recovered on 1.5% agarose gel. Lanes L represented the ladder, while other lanes for ERIC PCR products.

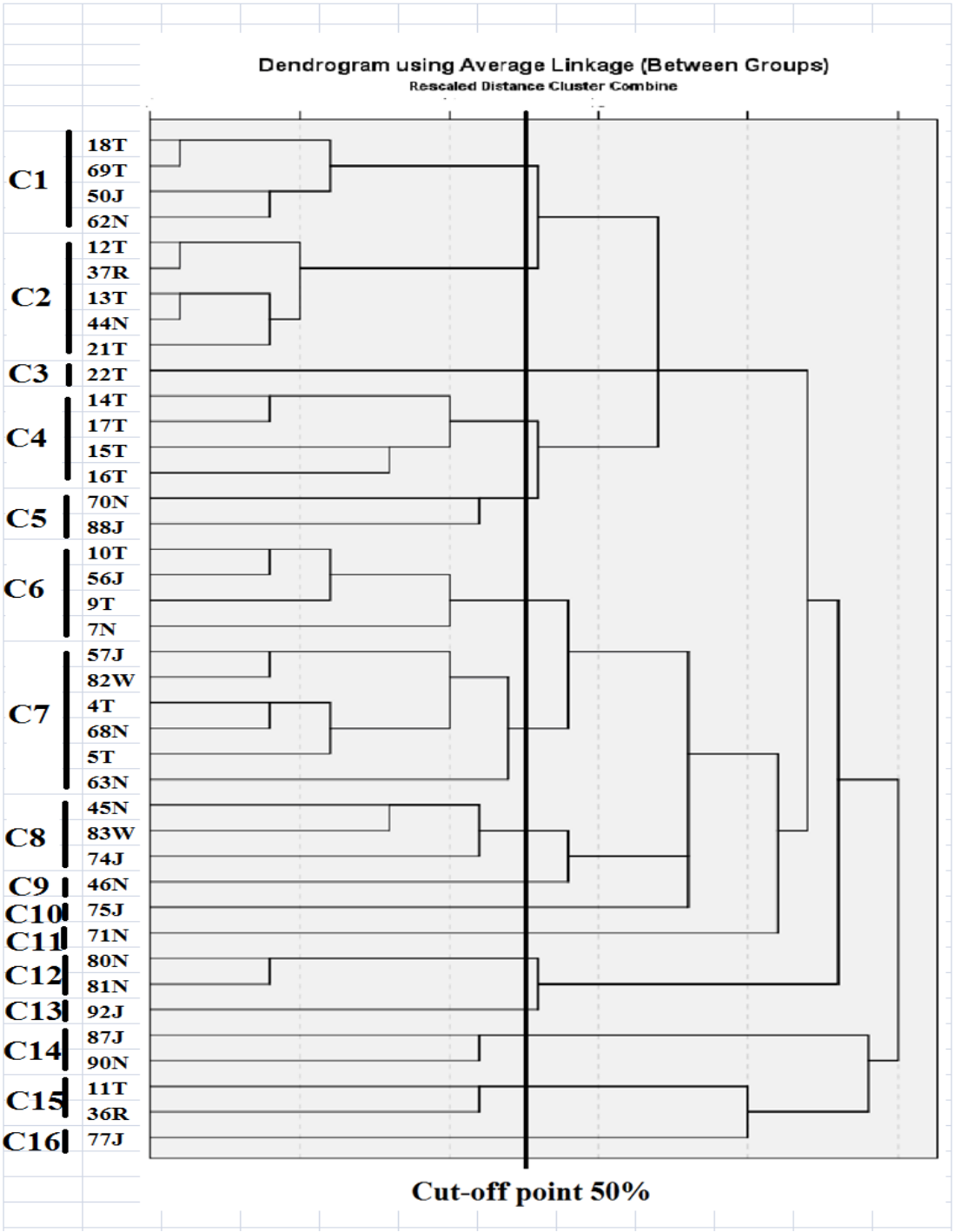


Figure 3.5. Dendrogram of 40 *K. pneumoniae* isolates carried genes for ESBLs, MBLs and/or AmpC β-lactamases based on the UPGMA method derived from analysis of the ERIC-PCR-profiles at a 50% similarity level.

C: Cluster

Chapter Four

Discussion

Complications of bacterial infection are considered as an important factor of outcome for patients seeking hospital admission specially in the intensive care units. In particular, this is true in the present period due to an increasing in resistance of antimicrobial agents that affects many microbial pathogens (Mainardi *et al*., 1998). *K. pneumoniae* is one of opportunistic pathogens that considerably cause hospital infections (Podschun and Ullmann, 1998). Beta-lactam antibiotics are broadly used in the treatment of bacterial infections, and this may lead a significant increase in the occurrence of β-lactamases-associated infections throughout the people in the world (Shaikh *et al*., 2014).

Detection of ESBL was based only on the PCR results, so the term possible ESBL was used. Results of this study showed that the prevalence of possible ESBL among clinical isolates of *K. pneumoniae* in Palestine is too high. In the Middle East countries, the prevalence of ESBL producers *K. pneumoniae* ranged from 17.4%-80% (Jarjees, 2006; Fam *et al*.,2006; Al-Zarouni *et al*., 2008; Bindayna *et al*., 2009; Al-Agamy *et al*., 2009; Batchoun *et al*., 2009; Mehrgan *et al*., 2010; Nedjai *et al*., 2012). In previous studies carried out in Jordan, results showed that the prevalence of ESBL among tested clinical *K. pneumoniae* isolates ranged from 38%-71.4% (Youssef *et al*.,1999; Shehabi *et al*., 2000; Batchoun *et al*., 2009). This may indicate that the prevalence of β-lactamases, particularly ESBL-producing microorganisms is increasing rapidly.

Result of the current research showed that TEM type ESBL was most common in these tested clinical *K. pneumoniae* isolates. Result of this

research is in agreement with other studies (Ghasemi *et al*., 2013; Chelliah *et al*., 2014). In contrast, other reports from different countries showed that CTX-M-type or SHV-type ESβL was most common (Yan *et al*., 2006; Daoud *et al.*, 2008; Heffernan *et al.*, 2009; Al-Agamy *et al*., 2009; Dzierzanowska *et al*., 2010; Nedjai *et al*., 2012). Sequence analyses and phylogenetic relationships of sequenced *TEM*, *SHV* and *OXA* genes showed that these genes belonged to *TEM-1*, *SHV-1* and *OXA-1* genes, and this result was consistent with various studies (Khosravi *et al*., 2013; Sugumar *et al*., 2014). Coexistence of two ESBL genes (*TEM* and *OXA*) was observed in clinical isolates of *K. pneumoniae* in this study. Presence of coexistence of ESBL genes in a single isolate was reported previously (Kiratisin *et al*., 2008; Denisuik *et al*., 2013).

Current study showed that 3.9% of *K. pneumoniae* isolates had AmpC β-lactamase genes. The prevalence of *AmpC* β- lactamase genes among clinical isolates of *K. pneumoniae* was reported from various parts of the world and had a range from 2.3% to 47% (Yan *et al*., 2006; Altun *et al*., 2013; Bareja *et al*., 2013; Oberoi *et al*., 2013; Mansouri *et al*., 2014; Gajul *et al*., 2015; Madhumati *et al.*, 2015). Low prevalence of *AmpC* β-lactamase genes detected among *K. pneumoniae* isolates in Palestine was due to that primers in this research used to amplified plasmid encoding genes only. Conventional methods used to detect *AmpC* β-lactamase enzymes have ability to detect both plasmid and non-plasmid-derived (chromosomal) AmpC activity (Adwan and Abu Jaber 2016). In this research, sequence analyses and phylogenetic relationships showed only

the DHA-1 subtype of AmpC β-lactamases was detected among these clinical isolates of *K. pneumoniae*. This result was in agreement with other studies (Lagha *et al*. 2014; Matsumura *et al*., 2015). Whereas, other studies from various parts of the world reported the presence of other different genes (Yan *et al*., 2004; Ktari *et al*., 2006; Yan *et al*., 2006; Chen *et al*., 2007; Shahid *et al*., 2009; Fam *et al*., 2013).

The increasing and rapid spread of MBL producing *Enterobacteriaceae*, particularly *K. pneumoniae* constitutes a serious medical problem to public health worldwide. Results of the current study showed that 9.8% of *K. pneumoniae* isolates carried MBL genes. Several recent reports from different places in the world demonstrated the prevalence rate of MBL production among clinical isolates of *K. pneumoniae* vary greatly and ranged from 8% to 53% (Enwuru *et al*., 2011; Wadekar *et al*., 2013; Vinodkumar *et al*., 2013; Oberoi *et al*., 2013; Bora *et al*., 2014; Ahmad and Ali, 2014; Gajul *et al*., 2015). Sequence analyses and phylogenetic relationships of NDM genes revealed the presence of NDM-1type gene, this report is considered the first one about detection of *NDM* gene in Palestine.

Emergence and spread of NDM-1 producing *K. pneumoniae* isolates has been reported in several studies around the world (Kumarasamy *et al*., 2010; Gaibani *et al*., 2011; Bhaskar *et al*., 2013; Shahcheraghi *et al*., 2013; Sonnevend *et al*., 2013; Lovayová *et al*., 2014). On the other hand, many other studies showed the presence of different subtypes of MBLs (Giakkoupi *et al*., 2003; Luzzaro *et al*., 2004; Roche *et al*., 2009; Gaibani *et*

al., 2011; Hoang *et al*., 2013; Shahcheraghi *et al*., 2013; Sonnevend *et al*., 2013; Lovayová *et al*., 2014).

Coexistence of two or three types of β-lactamase genes in single isolates was observed in this study. This will seriously restrict the options of antibiotics used in treatment, may create diagnostic problem and this may lead to use an unsuitable antimicrobial agent and the result may be fatal (Oberoi *et al*., 2013). Coexistence of more than one of β-lactamase classes or multiple genes of ESβLs, MBLs or AmpC β-lactamases has been reported widely from different species of bacterial pathogens including *K. pneumoniae* (Yan *et al*., 2004; Chen *et al*., 2007; Shahid *et al*., 2009; Jamal *et al*., 2013; Oberoi *et al*., 2013; Gajul *et al*., 2015).

In this study, a total of 51% of clinical isolates of *K. pneumoniae* carried class 1 integrons and none carried other classes of integons. This result is in agreement with other reports recently published, which showed that class 1 integrons was the most common or the only detected among clinical isolates of *K. pneumoniae* (Derakhshan *et al*., 2014; Lima *et al*., 2014; Mobarak-Qamsari *et al*., 2014). High prevalence of class 1 integrons among clinical *K. pneumoniae* isolates support high genetic variability and rapid dissemination of beta-lactamase genes and other coexisted genes among these isolates in hospitals (Lima *et al*., 2014).

Genotyping of β-lactamases-producing *K. pneumoniae* isolates by the ERIC-PCR technique showed different DNA profiles. At 50% similarity cut-off value analysis, there were a total of 16 groups or clusters among the tested 40 *K. pneumoniae* isolates. These findings indicate that isolates

included in the current study were genetically diverse and multiple clones of β-lactamase-producing *K. pneumoniae* isolates were prevalent in these hospitals. This clonal diversity suggests that most of the strains have been unable to be maintained or spread in different hospital settings. This observation challenges many conventional thoughts about the hospital epidemiology of antibiotic resistance including β-lactamase. This supporting that the high prevalence of β-lactamases-producing clinical *K. pneumoniae* isolates may be due to high selective pressure decreed by the high rate and misuse of antimicrobials particularly cephalosporin and its derivatives in this country could be the only major cause (Adwan and Abu Jaber, 2016).

To our knowledge, up to now, this is the first study documented the prevalence and molecular characterization of β-lactamases producing *K. pneumoniae* isolates in Palestine. In conclusion, our results showed high occurrence of β-lactamases among *K. pneumoniae* isolates in Palestine. Based on these results, the study recommends the continuous monitoring and surveillance of the prevalence, proper prevention practices and effective antibiotic use will restrict the further spread of β-lactamases producing *K. pneumoniae* isolates within hospitals in this country.

References

- Abbassi MS, Torres C, Achour W, Vinué L, Sáenz Y, Costa D, Bouchami O, Ben Hassen A. **Genetic characterisation of CTX-M-15-producing *Klebsiella pneumoniae* and *Escherichia coli* strains isolated from stem cell transplant patients in Tunisia.** Int J Antimicrob Agents 2008;32(4):308-314.
- Abbott S L. ***Klebsiella, Enterobacter, Citrobacter, Serratia, Plesiomonas,* and Other *Enterobacteriaceae*.** In P. R. Murray, E. J. Baron, J. H. Jorgensen, M. L. Landry & M. A. Pfaller (Eds.), Manual of Clinical Microbiology 2007;9th ed., pp. 698-711. Washington, USA: ASM Press.
- Adwan G, Abu Jaber A. **Frequency and molecular characterization of β-lactamases producing *Escherichia coli* isolated from North of Palestine.** Br Microbiol Res J 2016;11(5):1-13, Article no.BMRJ.22631.
- Adwan G, Adwan K, Jarrar N, Salama Y, Barakat A. **Prevalence of *seg, seh* and *sei* genes among clinical and nasal *Staphylococcus aureus* isolates.** Br Microbiol Res J 2013; 3(2): 139-149.
- Ahmad SS, Ali FA. **Detection of ESBL, AMPC and Metallo Beta-Lactamase mediated resistance in Gram- negative bacteria isolated from women with genital tract infection.** Eur Sci J 2014;10(9): 193-209. ISSN: 1857-7881 (Print) e- ISSN 1857-7431.

- Aktas Z, Bal C, Midilli K, Poirel L, Nordmann P. **First IMP-1-producing *Klebsiella pneumoniae* isolate in Turkey.** Clin Microbiol Infect 2006;12:695-696.
- Al-Agamy MH, Shibl AM, Tawfik AF. **Prevalence and molecular characterization of extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* in Riyadh, Saudi Arabia.** Ann Saudi Med 2009;29(4): 253–257.
- Al-Muharrmi Z, Rafay A, Balkhair A, Jabri AA. **Antibiotic combination as empirical therapy for extended spectrum Beta-lactamase.** Oman Med J 2008;23(2):78-81.
- Altun Ş, Tufan ZK, Yağcı S, Önde U, Bulut C. **Extended Spectrum Beta-lactamases, AmpC and Metallo Beta-lactamases in Emerging Multi-drug Resistant Gram-negative Bacteria in Intensive Care Units.** Open Access Scientific Reports 2013;2: 707. doi:10.4172/scientificreports.707.
- Al-Zarouni M, Senok A, Rashid F, Al-Jesmi S M and Panigrahi D. **Prevalence and Antimicrobial Susceptibility Pattern of Extended-Spectrum Beta-Lactamase-Producing *Enterobacteriaceae* in the United Arab Emirates.** Medical Principles and Practice Journal 2008;17:32-36.
- Ambler, R.P. **The structure of β-lactamases.** Philos Trans R Soc Lond B Biol Sci 1980; 289**:**321-331.
- Babini GS, Livermore DM. **Antimicrobial resistance amongst *Klebsiella* spp. collected from intensive care units in Southern and**

Western Europe in 1997-1998. J. Antimicrob Chemother 2000;45:183-189.

- Bareja R, Pottathil S, Shah RK, Grover PS, Singh VA. **Simultaneous detection of Extended-spectrum-β-lactamase, AmpC-β-lactamase and Metallo-β-lactamase in gram negative clinical isolates on a single plate.** Journal of Dental and Medical Sciences 2013; 6(2):74-77.
- Batchoun RG, Swedan SF, Shurman AM. **Extended spectrum β-Lactamases among Gram-negative bacterial isolates from clinical specimens in three major hospitals in Northern Jordan.** Int J Microbiol 2009, Article ID 513874. doi:10.1155/2009/513874
- Ben Achour N, Mercuri PS, Power P, Belhadj C, Ben Moussa M, Galleni M, Belhadj O. **First detection of CTX-M-28 in a Tunisian hospital from a cefotaxime-resistant *Klebsiella pneumoniae* strain.** Pathol Biol (Paris) 2009;57(5):343-348.
- Bhaskar MM, Anand R, Harish B N. **Prevalence of *bla*NDM-1 Producing Blood Isolates of *Escherichia Coli, Klebsiella* species and *Enterobacter* species in a Tertiary Care Centre in South India.** J Microbiol Res Rev 2013;1(6):61-68.
- Bhattacharjee A, Sen MR, Prakash P, Gaur A, Anupurba S. **Increased prevalence of extended spectrum β-lactamase producers in neonatal septicaemic cases at a tertiary referral hospital.** Indian J Med Microbiol 2008;26(4):356-360.

- Bindayna KM, Senok AC, Jamsheer AE. **Prevalence of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in Bahrain.** J Infect Public Health 2009;2(3):129–135.
- Bora A, Sanjana R, Jha BK, Mahaseth SN, Pokharel K. **Incidence of metallo-beta-lactamase producing clinical isolates of *Escherichia coli* and *Klebsiella pneumoniae* in central Nepal.** BMC Res Notes 2014;7:557.
- Boyd DA, Tyler S, Christianson S, McGeerA, Muller MP, the Canadian nosocomial infection surveillance program, Health Canada. **Complete nucleotide sequence of a 92-kilobase plasmid harbouring the CTX-M-15 extended-spectrum beta-lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada.** Antimicrob Agents 2004; 48(10): 3758–3764
- Brisse S, Grimont F, Grimont PAD. **The genus *Klebsiella*. In: Dworkin M, Falkow S, Rosenberg E, Schleifer K-H, Stackebrandt E (eds).** The Prokaryotes.2006; A Handbook on the Biology of Bacteria. 3rd edition, pp., 159-196. New York: Springer.
- Bush K, Jacoby GA. **Updated functional classification of β - lactamases.** Antimicrob Agents Chemother 2010;54**:**969-976.
- Bush K. Bench-to-bedside review: **The role of β-lactamases in antibiotic-resistant Gram-negative infections.** Crit Care 2010;14:224. doi: 10.1186/cc8892.

- Cappuccino JG, Sherman N. **Microbiology: A laboratory Manual.** 4th ed. The Binjamin/Cummings Publishing Company. Inc Californai. USA. 1996;pp: 477.
- Chaudhary M, Payasi A. **Antimicrobial susceptibility patterns and molecular characterization of *Klebsiella pneumoniae* clinical Isolates from North Indian patients.** Int J Med Med Sci 2013;46:7.
- Chelliah A, Thyagarajan R, Katragadda R, Leela KV, Babu RN. **Isolation of MRSA, ESBL and AmpC - β-lactamases from Neonatal Sepsis at a Tertiary Care Hospital.** J Clin Diagn Res. 2014;8(6):DC24-27.
- Chen YT, Lauderdale TL, Liao TL, Shiau YR, Shu HY, Wu KM, Yan JJ, Su IJ, Tsai SF. **Sequencing and comparative genomic analysis of pK29, a 269-Kilobase conjugative plasmid encoding CMY-8 and CTXM-3 β -Lactamases in *Klebsiella pneumoniae*. Antimicrob Agents Chemother** 2007; 51(8): 3004-3007.
- **Clinical Laboratory Standards Institute: Performance standards for antimicrobial susceptibility testing.** Twenty second informational supplement.2012; Wayne, PA, USA: CLSI: M100-S22.
- Daoud Z, Hobeika E, Choucair A, Rohban R. **Isolation of the first metallo-beta-lactamase producing *Klebsiella pneumoniae* in Lebanon.** Rev Esp Quimioter. 2008;21(2):123-126.
- Denisuik Andrew J., Lagace´-Wiens Philippe R. S., Pitout Johann D., Mulvey Michael R., Simner Patricia J., Tailor Franil, James A. Karlowsky James A., Hoban Daryl J., Adam Heather J. and Zhanel

George G. on behalf of the Canadian Antimicrobial Resistance Alliance (CARA). **Molecular epidemiology of extended-spectrum b-lactamase-, AmpC b-lactamase- and carbapenemase-producing Escherichia coli and *Klebsiella pneumoniae* isolated from Canadian hospitals over a 5 year period: CANWARD 2007–11.** J Antimicrob Chemother 2013; 68 Suppl 1: i57–i65

- Derakhshan S, Peerayeh SN, Fallah F, Bakhshi B, Rahbar M and Ashrafi A. **Detection of class 1, 2, and 3 integrons among *Klebsiella pneumoniae* isolated from children in Tehran Hospitals.** Arch Pediatr Infect Dis 2014 ; 2(1):164-168
- Dortet L, Poirel L, Nordmann P. **Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria.** Biomed Res Int 2014;2014:249856. doi: 10.1155/2014/249856.
- Dzierzanowska D, Kamińska W, Semczuk K, Borowiec D, Matysiak M, Szumała-Kakol A, Gierczyński R, Patzer JA. **Carriage of genes for various extended-spectrum beta-lactamases: a novel resistance strategy of *Klebsiella pneumoniae* in Poland.** Int J Antimicrob Agents. 2010;35(4):392-395.
- Ellington MJ, Kistler J, Livermore DM, Woodford N. **Multiplex PCR for rapid detection of genes encoding acquired metallo-β-lactamases.** J Antimicrob Chemother 2007; 59(2):321-322.
- Enwuru NV, Enwuru CA, Ogbonnia SO, Adepoju-Bello AA. **Metallo-β-Lactamase production by *Escherichia coli* and *Klebsiella* species**

isolated from hospital and community subjects in Lagos, Nigeria. Nat Sci 2011;9(11):1-5.

- Fam N, Gamal D, El Said M, El Defrawy I, El Dadei E, El Attar S, Sorur A, Ahmed S and Klena J. **Prevalence of plasmid-mediated *amp*C genes in clinical isolates of *Enterobacteriaceae* from Cairo, Egypt.** Br Microbiol Res J 2013;3(4):525-537.
- Fam N, Diab M, Helmi H, El- Defrawy I. **Phenoypic detection of metallo-β-Lactamases and extended spectrum β-Lactamases among Gram negative bacterial isolates.** Egypt J Med Microbiol 2006;15(4):719-730.
- Gaibani P, Ambretti S, Berlingeri A, Cordovana M, Farruggia P, Panico M, Landini MP, Sambri V. **Outbreak of NDM-1-producing *Enterobacteriaceae* in northern Italy.** Euro Surveil 2011;16(47):20027.
- Gajul SV, Mohite ST, Mangalgi SS, Wavare SM, Kakade SV. ***Klebsiella Pneumoniae* in Septicemic Neonates with Special Reference to Extended Spectrum β-lactamase, AmpC, Metallo β-lactamase Production and Multiple Drug Resistance in Tertiary Care Hospital.** J Lab Physicians 2015;7(1):32-37.
- Gandhi S, Ranjan KP, Ranjan N, Sapre N, Masani M**. Incidence of neonatal sepsis in tertiary care hospital: an overview.** Int J Med Sci Public Health 2013;2(3):548-552.
- Ghasemi Y , Archin T , Kargarm M , Mohkam M. **A simple multiplex PCR for assessing prevalence of extended-spectrum B-lactamases**

producing *Klebsiella pneumoniae* in Intensive Care Units of a referral hospital in Shiraz, Iran. Asian Pac J Trop Biomed 2013;703-708

- Giakkoupi P, Xanthaki A, Kanelopoulou M, Vlahaki A, Miriagou V, Kontou S, Papafraggas E, Malamou-Lada H, Tzouvelekis LS, Legakis NJ, Vatopoulos AC. **VIM-1 metallo-β-lactamase-producing *Klebsiella pneumoniae* strains in Greek hospitals.** J Clin Microbiol 2003;41:3893-3896.
- Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ. **Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics.** Antimicrob Agents Chemother. 2001;45(3):723-726.
- Gunseren F, Mamikoglu L, Ozturk S, Yucesoy M, Biberoglu K, Yulug N, Doganay M, Sumerkan B, Kocagoz S, Unal S, Cetin S, Calangu S, Koksal I, Leblebicioglu H, Gunaydin M. **A surveillance study of antimicrobial resistance of gram-negative bacteria isolated from intensive care units in eight hospitals in Turkey.** J. Antimicrob. Chemother 1999;43:373-378.
- Hammami S, Boutiba-Ben Boubaker I, Ghozzi R, Saidani M, Amine S, Ben Redjeb S. **Nosocomial outbreak of imipenem-resistant *Pseudomonas aeruginosa* producing VIM-2 metallo-β-lactamase in a kidney transplantation unit.** Diagn Pathol 2011; 6:106.

- Hanberger HJ, Garcia-Rodriguez A, Gobernado M, Goossens H, Nilsson LE, Struelens MJ. **Antibiotic susceptibility among aerobic gram-negative bacilli in intensive care units in 5 European countries. French and Portuguese ICU Study Groups.** JAMA 1999;281:67-71.
- Heffernan HM, Woodhouse RE, Pope CE, Blackmore TK. **Prevalence and types of extended-spectrum beta-lactamases among urinary *Escherichia coli* and *Klebsiella* spp. in New Zealand.** Int J Antimicrob Agents. 2009;34(6):544-549.
- Hill EB, Henry DA, Speert DP. In: Patric R.Murray, Ellen Jo Baron, James H Jorgensen, Marie Louise Landry, Michael A. Pfaller. **Manual of Clinical Microbiology,** 9th Edition, ASM Press, Washington, D.C. 2007; 734-48.
- Hoang TH, Wertheim H, Minh NB, Duong TN, Anh DD, Phuong TT, Son TH, Izumiya H, Ohnishi M, Shibayama K, Hien NT. **Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* strains containing New Delhi metallo-beta-lactamase isolated from two patients in Vietnam.** J Clin Microbiol 2013;51(1):373-374.
- Jacoby GA, Medeiros AA. **More extended spectrum β-lactamases. Antimicrob Agents.** Chemother 1991;35:1697-1704.
- Jacoby GA. **AmpC beta-lactamases.** Clin Microbiol Rev 2009, 22:161-182.

- Jain A, Roy I, Gupta MK, Kumar M, Agarwal SK. **Prevalence of ESBL producing gram negative bacteria in septicaemic neonates in a tertiary care hospital.** Indian J Med Microbiol 2003;52:421–425.
- Jamal W, Rotimi VO, Albert MJ, Khodakhast F, Nordmann P, Poirel L. **High prevalence of VIM-4 and NDM-1 metallo-β-lactamase among carbapenem-resistant *Enterobacteriaceae*.** J Med Microbiol 2013; 62:1239-1244.
- Janda J M, Abbott S L. **The Genera *Klebsiella* and *Raoultella*. *The Enterobacteria* 2006;** (2nd ed., pp. 115-129). Washington, USA: ASM Press.
- Jarjees R K. **Bacteriological study of the incidence of genitourinary tract infection in diabetic women in Erbil.** M.Sc. thesis. Erbil: University of Salahaddin. College of Science. Iraq.2006
- Johnson TR, Case CL. **Laboratory Experiments in the Microbiology.** 1998. 5th ed.
- Jouini A, Vinué L, Ben Slama K, Sáenz Y, Klibi N, Hammami S, Boudabous A, Torres C. **Characterization of CTX-M and SHV extended-spectrum β-lactamases and associated resistance genes in *Escherichia coli* strains of food samples in Tunisia.** J Antimicrob Chemother 2007;60:1137-1141.
- Khosravi AD, Hoveizavi H, Mehdinejad M. **Prevalence of *Klebsiella pneumoniae* Encoding Genes for CTX-M-1, TEM-1 and SHV-1 Extended-Spectrum Beta Lactamases (ESBL) Enzymes in Clinical**

Specimens. Jundishapur Journal of Microbiology. 2013; 6(10): e8256, DOI:10.5812/jjm.8256.

- Kim J, Jeon S, Lee B, Park M, Lee H, Lee J, Kim S. **Rapid detection of extended spectrum β-lactamase (ESBL) for *Enterobacteriaceae* by use of a multiplex PCR-based method.** Infect Chemother. 2009; 41(3): 181–184.
- Kiratisin P, Apisarnthanarak A, Laesripa C Saifon P. **Molecular characterization and epidemiology of extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* isolates causing health care-associated infection in Thailand, where the CTX-M family is endemic.** Antimicrob Agents Chemother 2008;52(8):2818-2824
- Koh TH, Babini GS, Woodford N, Sng LH, Hall LM, Livermore DM. **Carbapenem-hydrolysing IMP-1 β-lactamase in *Klebsiella pneumoniae* from Singapore.** Lancet 1999;353:2162.
- Kolar M, Latal T, Cermak P, Bartonikov N, Chmelarov E, Saue P, Kesselov M, on behalf of the Research Group. **Prevalence of extended-spectrum β-lactamase-positive *Klebsiella pneumoniae* isolates in the Czech Republic.** Int J Antimicrob Agents 2006; 28:49-53.
- Ktari S, Arlet G, Mnif B, Gautier V, Mahjoubi F, Ben Jmeaa M, Bouaziz M, Hammami A. **Emergence of Multidrug-Resistant *Klebsiella pneumoniae* isolates producing VIM-4 Metallo-β-Lactamase, CTX-M-15 Extended-Spectrum β -Lactamase, and**

CMY-4 AmpC β-Lactamase in a Tunisian University Hospital. Antimicrob Agents Chemother 2006; 50: 4198-4201.

- Kumarasamy K, Thirunarayan MA, Krishnan P. **Coexistence of blaOXA-23 with blaNDM-1 and armAin clinical isolates of *Acinetobacter* baumanii from India.** J Antimicrob Chemother 2010;65:2253–2254.
- Lagha N, Abdelouahid DE , Hassaine H , Robin F and Bonnet R. **First characterization of CTX-M-15 and DHA-1 β-lactamases among clinical isolates of *Klebsiella pneumoniae* in Laghouat Hospital, Algeria.** African Journal of Microbiology Research 2014; 8(11): 1221-1227.
- Leflon-Guibout V, Jurand C, Bonacorsi S, Espinasse F, Guelfi M C, Duportail F, Heym B, Bingen E and Nicolas-Chanoine M H. **Emergence and spread of three clonally related virulent isolates of CTX-M 15-producing *Escherichia coli* with variable resistance to aminoglycosides and tetracycline in a French geriatric hospital.** Antimicrob Agents Chemother 2004; 48, 3736–3742.
- Lima AM, de Melo ME, Alves LC, Brayner FA, Lopes AC. **Investigation of class 1 integrons in *Klebsiella pneumoniae* clinical and microbiota isolates belonging to different phylogenetic groups in Recife, State of Pernambuco.** Rev Soc Bras Med Trop 2014;47(2):165-169.
- Lincopan N, Leis R, Vianello MA, de Araújo MR, Ruiz AS, Mamizuka EM. **Enterobacteria producing extended-spectrum β-lactamases and**

IMP-1 metallo-β-lactamases isolated from Brazilian hospitals. J Med Microbiol 2006;55:1611-1613.

- Livermore DM, Brown DF, Quinn JP, Carmeli Y, Paterson DL, Yu VL: **Should third-generation cephalosporins be avoided against AmpC-inducible *Enterobacteriaceae*?** *Clin Microbiol Infect* 2004, 10:84-85.
- Lovayová V, Vargová L, Habalová V, Pastvová L, Curová K, Siegfried L. **New Delhi Metallo-Beta-Lactamase Ndm-1 Producing *Klebsiella Pneumoniae* in Slovakia.** Clin Microbial 2014;3:162. doi: 10.4172/2327-5073.1000162.
- Luzzaro F, Docquier JD, Colinon C, Endimiani A, Lombardi G, Amicosante G, Rossolini GM, Toniolo A. **Emergence in *Klebsiella pneumoniae* and *Enterobacter cloacae* clinical isolates of the VIM-4 metallo-β-lactamase encoded by a conjugative plasmid.** Antimicrob Agents Chemother 2004;48:648-650.
- Luzzaro F, Mantengoli E, Perilli M. **Dynamics of a nosocomial outbreak of multidrug-resistant *Pseudomonas aeruginosa* producing the PER-1 extended-spectrum beta lactamase.** J Clin Microbiol 2001; 39: 1865–1870.
- Madhumati B, Rani L, Ranjini C.Y and Rajendran R. **Prevalence of AMPC Beta Lactamases among Gram Negative Bacterial Isolates in a Tertiary Care Hospital.** Int. *J. Curr. Microbiol. App. Sci* 2015; 4(9): 219-227
- Majda Q, Najma A, Summyia B. **Evaluation of extended spectrum beta-lactamase mediated resistance in *Escherichia coli* and**

Klebsiella **in urinary tract infection at a tertiary care hospital.** Biomedica 2013;29:78-81.

- Mainardi JL, Carlet J, Acar J. 1998. **Antibiotic resistance problems in the critical care unit.** Crit Care Clin 1998;14:199-219.
- Mamlouk K, Boutiba-Ben Boubaker I, Gautier V, Vimont S, Picard B, Ben Redjeb S, Arlet G. **Emergence and outbreaks of CTX-M β-lactamase producing *Escherichia coli* and *Klebsiella pneumoniae* strains in a Tunisian hospital.** J Clin Microbiol 2006;44:4049-4056.
- Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. **Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe.** Antimicrob Agents Chemother. 2005;49:71-76.
- Mansouri S, Kalantar Neyestanaki D, Shokoohi M, Halimi S, Beigverdi R, Rezagholezadeh F, Hashemi A. **Characterization of AmpC, CTX-M and MBLs types of β-lactamases in clinical isolates of *Klebsiella pneumoniae* and *Escherichia coli* producing Extended Spectrum β-lactamases in Kerman, Iran.** Jundishapur J Microbiol 2014;7(2):e8756.
- Matsumura Y, Tanaka M, Yamamoto M, Nagao M, Machida K, Ito Y, Takakura S, Ogawa K, Yoshizawa A, Fujimoto Y, Okamoto S, Uemoto S, **,**Ichiyama S. **High prevalence of carbapenem resistance among plasmid-mediated AmpC β-lactamase-producing *Klebsiella pneumoniae* during outbreaks in liver transplantation units.** Int J Antimicrob Agents 2015;45(1):33-40

- Mazel, D. **Integrons: agents of bacterial evolution.** Nat Rev Microbiol 2006; 4: 608-620.
- Mehrgan H, Rahbar M, Arab-Halvaii Z. **High prevalence of extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a tertiary care hospital in Tehran, Iran.** J Infect Dev Ctries 2010;4(3):132-138.
- Messai L, Achour W, Ben Hassen A. **Epidemiological profile of enterobacteria isolated from neutropenic patients.** Pathol Biol (Paris). 2007;55(5):230-234. [Article in French]
- Mobarak-Qamsari M, Ashayeri-Panah M, Eftekhar F, Feizabadi MM. **Integron mediated multidrug resistance in extended spectrum beta-lactamase producing clinical isolates of *Klebsiella pneumoniae*.** Braz J Microbiol 2014;44(3):849-854.
- Modi DJ, Patel BV, Patel MH, Bhatt SS, Sood NK, Vegad MM. **A study of extended spectrum β-lactamase (ESBL) and AmpC β-lactamase producing *Klebsiella pneumoniae* in neonatal intensive care unit at tertiary care hospital, Ahmadabad.** Natl J Community Med. 2012;3:523–528.
- Monstein H., Ostholm-Balkhed A., Nilsson M., Nilsson M., Dornbusch K. and Nilsson L. **Multiplex PCR amplification assay for the detection of *bla*SHV, *bla*TEM and *bla*CTX-M genes in *Enterobacteriaceae*.** APMIS: Acta pathologica, microbiologica, et immunologica Scandinavica 2007; 115**:**1400-1408.

- Nathisuwan S, Burgess DS, Lewis JS II. **Extended-spectrum β-lactamases: epidemiology, detection, and treatment.** Pharmacotherapy 2001;21(8):920- 928.
- Nedjai S, Barguigua A, Djahmi N, Jamali L, Zerouali K, Dekhil M, Timinouni M. **Prevalence and characterization of extended spectrum β-lactamases in *Klebsiella-Enterobacter-Serratia* group bacteria, in Algeria.** Médecine et maladies infectieuses 2012;42:20-29.
- Neuhauser MM, Weinstein RA, Rydman R, Danziger LH, Karam G, Quinn JP. **Antibiotic resistance among gram-negative bacilli in US intensive care units: Implications for fluoroquinolone use.** JAMA 2003; *289*, 885–888.
- Oberoi L, Singh N, Sharma P, Aggarwal A. **ESBL, MBL and AmpC β Lactamases Producing Superbugs - Havoc in the Intensive Care Units of Punjab India.** J Clin Diagn Res 2013;7(1):70-73.
- Otman J, Cavassin ED, Perugini ME, Vidotto MC. **An outbreak of extended-spectrum beta-lactamase-producing *Klebsiella* species in a neonatal intensive care unit in Brazil.** Infect Control Hosp Epidemiol 2002;23:8–9.
- Partridge SR, Collis CM, Hall RM. **Class 1 integron containing a new gene cassette, *aadA10*, associated with Tn1404 from R151.** Antimicrob Agents Chemother 2002; 46: 2400–2408.
- Paterson DL, Bonomo RA. **Extended-spectrum β-lactamases: A clinical update.** Clin Microbiol Rev. 2005;18:657-686.

- Paterson DL, Hujer KM, Hujer AM, Yeiser B, Bonomo MD, the International *Klebsiella* study group. **Extended spectrum beta-lactamases in *Klebsiella pneumoniae* bloodstream isolates from seven countries: dominance and widespread prevalence of SHV-and CTX-M type beta-lactamases.** Antimicrob Agents Chemother 2003:47**:** 3553-3560.
- Paterson DL. **Recommendation for treatment of severe infections caused by *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBLs).** Clin Microbiol Infect 2000;6:460-463.
- Pérez-Pérez FJ, Hanson ND. **Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR.** J Clin Microbiol 2002;40(6):2153-2162.
- Peterson DL. **Resistance in gram- negative bacteria : *Enterobacteriaceae.*** Am J Infect Control 2006;34: S20 –S28.
- Pfaller MA, Jones RN, Doern GV. **Multicenter evaluation of the antimicrobial activity for six broad-spectrum beta-lactams in Venezuela: comparison of data from 1997 and 1998 using the E test method. Venezuelan Antimicrobial Resistance Study Group.** Diagn Microbiol Infect Dis 1993;35:153–158.
- Pitout JD, Nordmann P, Kevin B, Laupland KB, Poirel L. **Emergence of *Enterobacteriaceae* producing extended-spectrum β-lactamases (ESBLs) in the community.** J Antimicrob Chemother 2005;56:52-59.

- Podschun R, Ullmann U. ***Klebsiella*** **spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors.** Clin Microbiol Rev 1998;11:589-603.
- Poirel L, Walsh TR, Cuvillier V, Nordmann P. **Multiplex PCR for detection of acquired carbapenemase genes.** Diagn Microbiol Infect Dis. 2011;70(1):119-123.
- Queenan AM, Bush K. **Carbapenemases: the versatile β-lactamases.** Clin Microbiol Rev 2007; 20:440-458.
- Recchia GD, Hall RM. **Gene cassettes: a new class of mobile element.** Microbiol 1995; 141: 3015–3027.
- Roche C, Cotter M, O'Connell N, Crowley B. **First identification of class A carbapenemase-producing *Klebsiella pneumoniae* in the Republic of Ireland.** Euro Surveill 2009;14(13):19163.
- Sader HS, Gales AC, Granacher TD, Pfaller MA, Jones RN. **Prevalence of antimicrobial resistance among respiratory tract isolates in Latin America: results from SENTRY antimicrobial surveillance program (1997-98)**. Braz J Infect Dis 2000;4:245–254.
- Senda K, Arakawa Y, Ichiyama S, Nakashima K, Ito H, Ohsuka, Shimokata K, Kato N, Ohta M. **PCR detection of metallo-β-lactamase gene (blaIMP) in gram-negative rods resistant to broad-spectrum beta-lactams.** J Clin Microbiol 1996;34:2909-2913.
- Shahcheraghi F, Nobari S, Rahmati Ghezelgeh F, Nasiri S, Owlia P, Nikbin VS, Imani Fooladi AA. **First Report of New Delhi Metallo-**

Beta-Lactamase- 1-Producing *Klebsiella pneumoniae* in Iran. Microb Drug Resist 2013;19(1):30-36.

- Shahid M, Ensor VM, Hawkey PM. **Emergence and dissemination of *Enterobacteriaceae* with plasmid-mediated CMY-6 and CTXM-15 beta-lactamases in community in North-India.** World J Microbiol Biotechnol 2009; 25: 1439-1446.
- Shaikh S, Fatima J, Shakil S, Rizvi SMD, Kamal MA. **Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment.** Saudi J Biol Sci 2014;22:90-101.
- Shehabi A A, Mahafzah A, Baadran I, Qadar F A, and Dajani N. **High incidence of *Klebsiella pneumoniae* clinical isolates to extended-spectrum β-lactam drugs in intensive care units.** Diagn Microbiol Infect Dis 2000;36(1):53-56.
- Shibata N, Doi Y, Yamane K, Yagi T, Kurokawa H, Shibayama K, Kato H, Kai K, Arakawa Y. **PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron.** J Clin Microbiol 2003; 41(12):5407-5413.
- Sonnevend A, Al Baloushi A, Ghazawi A, Hashmey R, Girgis S, Hamadeh MB, Al Haj M, Pál T. **Emergence and spread of NDM-1 producer *Enterobacteriaceae* with contribution of IncX3 plasmids in the United Arab Emirates.** J Med Microbiol 2013; 62:1044-1050.
- Spellberg B, Guidos R, Gilbert D, Bradley J, Boucher HW, Scheld WM, Bartlett JG, Edwards J. **Infectious Diseases Society of America. The**

epidemic of antibiotic-resistant infections: a call to action for the medical community from the Infectious Diseases Society of America. Clin Infect Dis 2008;46(2):155-164.

- Struve C, Krogfelt KA. **Pathogenic potential of environmental *Klebsiella pneumoniae* isolates.** Environ Microbiol 2004;6(6):584-590.
- Sugumar M, Kumar KM, Manoharan A, Anbarasu A, Ramaiah S. **Detection of OXA-1 β-lactamase gene of *Klebsiella pneumoniae* from blood stream infections (BSI) by conventional PCR and in-silico analysis to understand the mechanism of OXA mediated resistance.** PLoS One. 2014;9(3):e91800. doi: 10.1371/journal.pone.0091800.
- Tzelepi E, Magana Ch, Platsouka E, Sofianou D, Paniara O, Legakis NJ, Vatopoulos AC, Tzouvelekis LS. **Extended-spectrum beta-lactamase types in *Klebsiella pneumoniae* and *Escherichia coli* in two Greek hospitals.** Int J Antimicrob Agents. 2003;21(3):285-288.
- Vatopoulos A. **High rates of metallo-beta-lactamase-producing *Klebsiella pneumoniae* in Greece - a view of the Current evidence.** Eurosurveillance 2008;13(4)· pii: 8023.
- VinodKumar CS, Prasad BS, Kalapannavar NK, Raghu Kumar KG, Yogeesha BK, Jayasimha VL, Basavarajappa KG. **Carbapenemases mediated resistance among the isolates of neonatal septicemia.** J Public Health Med Res. 2013;1:24-27.

- Wadekar MD, Anuradha K, Venkatesha D. **Phenotypic detection of ESBL and MBL in clinical isolates of *Enterobacteriaceae*.** Int J of Curr Res Acad Rev 2013; 1(3):89-95.
- Walsh TR, Toleman MA, Poirel L, Nordmann P. **Metallo-β-lactamases: the quiet before the storm?** Clin Microbiol Rev 2005; 18:306-325.
- Wang M, Sahm DF, Jacoby GA, Hooper DC. **Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States.** Antimicrob Agents Chemother 2004;48:1295-1299.
- Winokur PL, Canton R, Casellas JM, Legakis N. **Variations in the prevalence of strains expressing an extended-spectrum β-lactamase phenotype and characterization of isolates from Europe, the Americas, and the Western Pacific region.** Clin Infect Dis 2001;32(Suppl 2):S94-S103.
- Woodford N, Dallow JW, Hill RL, Palepou MF, Pike R, Ward ME, Warner M, Livermore DM. **Ertapenem resistance among *Klebsiella* and *Enterobacter* submitted in the UK to a reference laboratory.** Int J Antimicrob Agents 2007;29:456-459.
- Yan JJ, Hsueh PR, Lu JJ, Chang FY, Shyr JM, Wan JH, Liu YC, Chuang YC, Yang YC, Tsao SM, Wu HH, Wang LS, Lin TP, Wu HM, Chen HM, Wu JJ. **Extended-spectrum β-lactamases and plasmid-mediated AmpC enzymes among clinical isolates of *Escherichia coli***

and *Klebsiella pneumoniae* from seven medical centres in Taiwan. Antimicrob Agents Chemother 2006; 50: 1861-1864.

- Yan JJ, Ko WC, Wu HM, Tsai SH, Chuang CL, Wu JJ. **Complexity of *Klebsiella pneumoniae* isolates resistant to both cephamycins and extended-spectrum cephalosporins at a teaching hospital in Taiwan.** J Clin Microbiol 2004; 42: 5337-5340.
- Youssef MT, Malkawi HI, Shurman AA, and Andremont AO. **Molecular typing of multiresistant *Klebsiella pneumoniae* isolated from children from northern Jordan.** Journal of Tropical Pediatrics, 1999;vol. 45, no. 5, pp. 271–277.
- Zulkifli Y, Alitheen NB, Son R, Raha AR, Samuel L, Yeap SK and Nishibuchi M. **Random amplified polymorphic DNA-PCR and ERIC PCR analysis on *Vibrio parahaemolyticus* isolated from cockles in Padang, Indonesia.** International Food Research Journal. 2009;16:141-150.

جامعة النجاح الوطنية
كلية الدراسات العليا

الوصف الجزيئي ومدى انتشار النوع البكتيري كليبسيلا الرئوية المنتجة للإنزيمات المحللة للمضادات الحيوية من نوع بيتا لاكتام في شمال فلسطين

إعداد
دعاء فيصل ربايعة

إشراف
د. غالب عدوان

قدمت هذه الأطروحة استكمالاً لمتطلبات الحصول على درجة الماجستير في العلوم الحياتية، بكلية الدراسات العليا، في جامعة النجاح الوطنية، في نابلس، فلسطين.
2016

الوصف الجزيئي ومدى انتشار النوع البكتيري كليبسيلا الرئوية المنتجة للإنزيمات المحللة للمضادات الحيوية من نوع بيتا لاكتام في شمال فلسطين في شمال فلسطين

إعداد

دعاء فيصل ربايعة

إشراف

الدكتور غالب عدوان

الملخص

تم الحصول على 51 عزلة من بكتيريا الكليبسيلا من مستشفيات مختلفة من منطقة جنين، نابلس وطولكرم/ فلسطين خلال الفترة ما بين أيلول إلى كانون الأول من العام 2015 . أظهرت النتائج أن انتشار كل من ESβL، MBL و AmpC β-lactamase باستخدام تقنية PCR كان بنسبة 92.2%، 9.8% و 3.92% على الترتيب. كما أظهرت النتائج أن جين *TEM* كان سائدا بنسبة 72.5% بين بكتيريا الكليبسيلا التي تحمل جينات ESβL. أما الجينات الأخرى وهي *CTX-M* ، *SHV* و *OXA* فكانت النسب 0.0%،17.6 % و31% على الترتيب. بينما في AmpC β-lactamases فقد تم تحديد جين *DHA* فقط، وكانت نسبة انتشاره 3.9% . وأظهرت النتائج أن العينات المنتجة ل MBL كانت تحمل جين NDM بنسبة انتشار 9.8%. بالإضافة إلى أن 50.98% من العينات كانت تحمل النوع 1 من integrons ولم يظهر النوع 2، 3 أو4 في أي من العينات. أظهر التحليل الجزيئي باستخدام طريقة شجرة النشوء والتطور أن جميع تسلسلات جينات *TEM*، *SHV*، *OXA*، *NDM* و *DHA* التي تم فحص تسلسل النيوكليتيدات لها تعود إلى *TEM-1* ، *SHV-1* ، *OXA-1* ،NDM-1 و *DHA-1* على الترتيب. كما أظهرت نتائج ERIC-PCRأن هذه السلالات متنوعة.

نتائجنا تؤكد على الحاجة إلى المراقبة المستمرة ومراقبة الانتشار والرقابة السليمة وممارسات الوقاية واستخدام المضادات الحيوية الفعالة سيحد من انتشار المزيد من السلالات المنتجة ل AmpC β-lactamases، ESβLs و MBLs داخل المستشفيات في فلسطين.