Molecular Characterization of Carbapenemases and Quinolone Resistance
Determining Region Enzymes-Producing Isolates in an Outbreak at the University Hospital of Leipzig
Dissertation
Zur Erlangung des akademischen Grades Dr.rer.med.
an der Medizinischen Fakultät
der Universität Leipzig
eingereicht von:
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Beschluss über die Verleihung des Doktorgrades vom:

21.10.2014
Dedication

I lovingly dedicate this thesis to my husband for his remarkable patience and unwavering love and support each step of the way and for the days of daddy daycare to our beautiful, Zeena, during my final days before defending my thesis.

A special feeling of gratitude to my loving parents Burhan and Ameera; who encouraged me until the end, to my sister Hana and my brothers Mohammad, Ameer, and Zaid.

I also dedicate this thesis to my baby daughter Zeena.
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List of Abbreviations

- KPCs: *Klebsiella pneumoniae* Carbapenemases
- PCR: Polymerase Chain Reaction
- PFGE: Pulsed Field Gel Electrophoresis
- QRDRm: Quinolone Resistance Determining Region mutations
- QNR: Quinolone Resistance
- EUCAST: European Committee on Antimicrobial Susceptibility Testing
- MIC: Minimum Inhibitory Concentration
- OXA: Oxacillinase
- VIM: Verona Integron-encoded Metallo-β-lactamase
- IMP: Imipenemase
- NDM-1: New Delhi Metallo-β-lactamase-1
  - aac-6-Ib: Aminoglycoside N-Acetyltransferase (6’) Ib
- MMWR: CDC-Morbidity and Mortality Weekly Report
- UTI: Urinary Tract Infection
This study aims to determine the prevalence and types of quinolone resistance and carbapenemases genes among different isolates from patients admitted to the University Hospital of Leipzig over a period of ten months.

During the period from January 2011 through October 2011, a total of 50 carbapenemases isolates were recovered from patients of the University Hospital of Leipzig/ Germany. The
KPC, IMP, VIM, OXA-48, NDM-1, and aac-6-Ib genes as well as qnrA, qnrB, and qnrS genes were detected by multiplex PCR, respectively.

Results showed that KPC gene was detected in 82% of the isolates. The qnrA, qnrS, IMP, NDM-1, and OXA-48 genes were not detected in any of the isolates while qnrB and VIM genes were found in 2%. On the other hand, aac-6-Ib gene was the most prevalent gene among the study isolates and composed a percentage of 96%. 
Molecular Characterization of Carbapenemases and Quinolone Resistance Determining Region Enzymes-Producing Isolates in an Outbreak at the University Hospital of Leipzig

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Abstract

Beta lactam resistance producing isolates of Enterobacteriacea and non-Enterobacteriacea have emerged since more than seventy years ago (Abraham and Chain, 1940). They are known to cause both community and hospital-acquired infections. Resistance against carbapenem is primarily mediated by the production of enzymes that destroy the beta lactam antimicrobials, which are produced by these isolates involving the expression of serine and metalobetalactamase genes KPC, IMP, VIM, NDM-1 and OXA-48. Quinolone resistance is predominantly mediated by mutations in the qnrA, qnrB, qnrS, and aac-6-Ib genes. Carbapenemase-producing organisms especially Klebsiella pneumoniae carbapenemases (KPCs) emerged as important pathogens especially among critically ill patients causing significant morbidity and mortality. This study aims to determine the prevalence and types of
quinolone resistance and carbapenemases genes among different isolates from patients admitted to the University Hospital of Leipzig over a period of ten months.

During the period from January 2011 through October 2011, a total of 50 carbapenemases isolates were recovered from patients of the University Hospital of Leipzig/ Germany. The isolates were identified by biochemical tests and their susceptibility to antimicrobials was determined by the microbroth dilution method according to ISO standard. The KPC, IMP, VIM, OXA-48, NDM-1, and aac-6- Ib genes as well as qnrA, qnrB, and qnrS genes were detected by multiplex PCR, respectively.

Results showed that KPC gene was detected in 82% of the isolates while 8% were KPC negative. The qnrA, qnrS, IMP, NDM-1, and OXA-48 genes were not detected in any of the isolates while qnrB and VIM genes were found in 2%. On the other hand, aac-6- Ib gene was the most prevalent gene among the study isolates and composed a percentage of 96%. Results also showed that KPC, and aac-6- Ib genes were detected in isolates collected from urine, blood, wounds, swabs, sputum, tracheal secretions, biopsies, and anal smears, while VIM gene was detected in one isolate collected from blood. The qnrB gene was found in one isolate collected from urine specimen.

The wide spread of carbapenem and quinolone resistance-producing organisms is a critical problem that complicates the treatment of infections resulting from these organisms. Necessary measures must, therefore, be taken to limit their spread, which include appropriate antibiotic treatment, control of hospital infections, observe of personal hygiene, and the use of appropriate methods of sterilization and disinfection to prevent the dissemination of these organisms.

**Keywords:** Resistance, carbapenemases, QRDR, multiplex PCR, antimicrobials
1 Introduction

1.1 Carbapenemases and Quinolone Resistance Determining Region mutations (QRDRm)

1.1.1 Literature Review

Resistance to β-lactam agents has been reported as early as 1940 (Abraham and Chain, 1940). Carbapenem resistance is of a special importance and produced by enzymes called carbapenemases (Queenan and Bush, 2007). The initial description of carbapenemase production was at the beginning of 1980s by a strain of Serratia marcescens in the United Kingdom and the United states (Yang et al., 1990, Naas T et al., 1994).

These enzymes can hydrolyze penicillins, cephalosporins and carbapenems and are inhibited by clavulanic acid (Queenan and Bush, 2007). Infections caused by carbapenemases such as KPCs become established worldwide such as in United states, countries in South America, Europe, Israel, Germany and Greece (Paterson & Bonomo, 2005, Vatopoulos, 2008, Kitche et al., 2009, Nordmann et al., 2009, Leavitt et al., 2010, Steinmann et al., 2011). In Asia, KPC was reported in China (Wei et al., 2007) Korea (Rhee et al., 2010) and Tiwan (Chung et al., 2011). Commonly, carbapenemase producing strains are also quinolone resistant (Yigit et. al., 2001, Bratu (C) et. al., 2005, Lomaestro et. al., 2006). Bacterial resistance should be a concern for physicians, it often results in treatment failure and leads to serious health problems (CDC, 2009, Borer et al., 2009, Daikos et al., 2009, Patel et al., 2008, Bratu (B)., 2005).

The quinolone resistance was found to be related to mutations that lead to amino acid alterations in the quinolone-resistance determining regions (QRDRs) within the subunits which are involved in the DNA synthesis. These subunits inhibit the DNA gyrase; the topoisomerases II which is responsible for GyrA and GyrB genes, and topoisomerase IV
which is responsible for ParC and ParE genes that end with the survival of the bacteria (Hooper, 1999).

The quinolone resistance genes (QnrS) as QnrA, QnrB, and QnrS were also reported as inhibitor for the DNA gyrase and showed resistance to nalidixic acid (Martinez et. al., 2003). Furthermore, the presence of a novel gene, aac(6’)-Ib-cr encoding a fluoroquinolone-modifying enzyme (aminoglycoside acetyltransferase) have been detected as an important gene which plays a significant role in the mechanism of quinolone resistance (Machado et. al., 2006).

The carbapenemases and QRDR genes are i.e found in K. pneumoniae; Escherichia coli (Carl et al., 2008), Pseudomonas aeruginosa (Villegas et.al., 2007), Shigella flexneri (Iyobe et al., 2000), Citrobacter spp (Chu et al., 2001), Klebsiella oxytoca (Kristóf et al., 2010), Enterobacter cloacae (Lee et al., 2008), Serratia marcescens (Lambert et al., 1994), Salmonella enterica (Cheung et al., 2005), and most recently in an Acinetobacter baumannii strain (Robledo et.al., 2010).

Resistance has a great potential for spreading among the organisms by extra chromosomal elements such as plasmids (Kang et al., 2005, Schwaber & Carmeli,2008, Queenan and Bush, 2007) as well as transposons, and the gene cassettes (Kang et al., 2005, Schwaber & Carmeli,2008). On the other hand, long-term care facilities and hospitalisation facilitate the spread of these organisms among people (Urban et al., 2008). Patients with unrecognized KPCs served as reservoirs for transmission during health care associated outbreaks (Samra et al., 2007). Early detection of carbapenemases and QRDR-producing organisms and introduction of strict infection control including hand hygiene and contact precautions can help control the spread of these organisms (Samra et al., 2007).

1.2 Clinical Impact of Multiresistance

Resistant may spread and pose broader infection-control problems in hospitals and community (Bruinsma et al., 2003). Increased mortality and morbidity rates have been
documented in multiple studies with different organisms in different specimen sites (Borer et al., 2009, Daikos et al., 2009, Patel et al., 2008, Bratu (B), 2005). Furthermore, children could be affected and the infections could be transmitted among the family members and among the students in schools (Garau et al., 1999). Finally, infections caused by carbapenemase producing organisms have been associated with increased treatment cost and hospital stay length (CDC, 2009).

1.3 Mechanisms of Antibiotic Resistance

Figure (1) summerizes the site of action for the known antibiotics and the mechanisms of antibiotic resistance.

![Antibiotics: sites of action and Mechanisms of resistance](Image)

Figure 1: Antibiotics; sites of action and Mechanisms of resistance. Modified with permission from the American Association for the Advancement of Science (Science1992;257:1064–73).3 Image by: Lianne Friesen and Nicholas Woolridge

1.3.1 Carbapenemases

Beta lactams antibiotics consist of all antibiotic agents that contain a β-lactam nucleus in its molecular structure. This includes penicillins, cephalosporins, monobactams, and
carbapenems (Holten and Onusko, 2000). They work by inhibiting bacterial cell wall synthesis. Bacteria often develop resistance to β-lactam antibiotics by beta-lactamase enzymes synthesis (such as carbapenemases), enzymes that attack the β-lactam ring. They may be inhibited by β-lactamase inhibitors such as clavulanic acid (Fig: 2, 3, and 4) (Hawkey, 1998; Wiegand et al., 2007). Carbapenemases include KPCs, VIM, IMP, OXA-48, and NDM-1.

Figure 2: Beta lactam ring destroyed by beta-lactamases enzymes
Figure 3: beta-lactamases enzymes coded for bla genes attacking beta-lactam antibiotics (David H., 2013).

Figure 4: Clavulanic acid inhibiting beta-lactamases

Beta-lactamase enzymes destroy the beta-lactam ring by two major mechanisms of action:

1. In the most common mechanism, beta-lactamases have a serine based mechanism of action. They are divided into two major classes in carbapenemases (A and D) on the basis of amino acid sequence (class A: KPC, class D: OXA-48). They contain the active site consisting of a narrow longitudinal groove, with a cavity on its floor, which
is loosely constructed in order to have conformational flexibility in terms of substrate binding (Fig: 5) (Bisson et al., 2002). Close to this, lies the serine residue that irreversibly reacts with the carbonyl carbon of the beta-lactam ring resulting in an open ring (inactive beta-lactam) and regeneration of the beta-lactamase. (Fluit et al., 2001; Page, 2002).

Figure 5: The Molecular structure of serine based carbapenemases (Jawahar Swaminathan and MSD staff at the European Bioinformatics Institute).

2. A less commonly group of beta-lactamases are the metallo beta-lactamases or class B beta-lactamases (Class B carbapenemases: IMP, VIM, and NDM-1). These use a divalent transition metal ion, most often zinc, linked to histidine or cysteine residue or both, to react with the carbonyl group of the amide bond of most penicillins, cephalosporins, but not monobactams (Fig: 6) (Fluit et al., 2001; Page, 2002)
1.3.2 QRDR

Since the mid 1990s quinolone resistance started to increase in bacteria (Dalhoff, 1999). Fluoroquinolones are the only class of antimicrobial agents in clinical use that inhibit the bacterial DNA synthesis. They inhibit two bacterial enzymes: DNA gyrase and topoisomerase IV, which have essential roles in DNA replication (Higgins et al., 2003). The quinolones bind to the complex of each of these enzymes with DNA; the resulting topoisomerase-quinolone-DNA ternary complex subsequently leads to a breaking DNA which ends with blocking the DNA replication enzyme complex. Ultimately, this action results in damage to bacterial DNA and bacterial cell death (Drlica et al., 2008; Drlica and Zhao et al., 1997, Hooper, 1999). The two main mechanisms of quinolone resistance are chromosomally encoded, being either modification of the quinolone targets with changes of DNA gyrase (gyrA) and/or topoisomerase IV (parC) genes that alter the conformation of target amino acid residues within the protein, or decreased intracellular concentration due to impermeability of the
membrane or overexpression of efflux pump systems (Oktem et al., 2008; Dalhoff, 2012; Allou et al., 2009; Poirel et al., 2006; Jacoby, 2005; Hernández et al. 2011).

In addition to the chromosomal mutations in the QRDRs, plasmid-mediated quinolone resistance (PMQR) determinants may also reduce the levels of quinolone susceptibility (Martinez-Martinez et al. 1998, Robicsek et al. 2006, Hernández et al. 2011).

The plasmid-mediated (transferable) fluoroquinolone resistance include several mechanisms:

1. Qnr, 2. Aminoglycoside acetyl-transferase AAC(6’)-Ib-cr and other mechanisms (Tran and Jacoby, 2002; Poirel et al., 2008; Robicsek et al., 2006; Strahilevitz et al., 2009; Poirel et al., 2012; Rodríguez-Martínez et al., 2011). The Qnr proteins protect DNA from quinolone binding to gyrase and topoisomerase IV (Martinez-Martinez et al. 1998; Tran and Jacoby, 2002; Nordmann and Poirel, 2005). The aac(6’)-Ib gene catalyzes acetylation of aminoglycosides while the aac(6’)-Ib-cr gene (a variant from aac(6’)-Ib gene) catalyzes acetylation of aminoglycosides and fluoroquinolone (Vetting et al., 2008).

1.3.3 Mode of Carbapenemases and QRDR Genes Transfer (Plasmid and Chromosomal)

The rapid spread and growing list of pathogens in which the resistant genes (such as KPC) have been isolated is probably due to its carriage mainly on plasmids (Jacoby and Bush, 2005; Nass et al., 2008; Medeiros, 1997). Plasmids are the major vehicle for the spread of bacterial resistance, as they can be transferred between Gram negative bacteria by conjugation (Samaha-Kfoury and Araj, 2003) (Fig: 7). The transfer of resistance genes are not only plasmid mediated but also chromosomal (Levesque et al., 1995).
1.3.4 Control the Spread of Beta Lactamases

To minimize the spread of beta lactamases, firstly, infection control practices efforts should be done by early identification of beta lactamases producing bacteria with in vitro testing (Todar, 2008, CDC, 2009), detecting colonization and preventing horizontal spread (Kochar et al., 2009). Secondly, standard precautions, especially hand hygiene and instruments sterilization should be observed (Todar, 2008). Furthermore, early detection of carriers; which include patients who were hospitalized and then transferred to another country, and the patients at risk such as patients who were in ICUs, patients who had transplantation operations, and immunocompromised patients (Miriagou et al., 2010, Nordmann et al., 2011).
1.3.5 Classification of Carbapenemases

For β-lactamases two major classifications are being used: the Ambler molecular classification and the Bush-Jacoby functional classification (Ambler, 1980; Bush, 1988; Bush et al., 1995; Rasmussen and Bush, 1997). And two major molecular families: metallo-carbapenemases and serine carbapenemases which are distinguished by hydrolytic mechanism at the active site (Bush et al., 1995). Metallo enzymes found to be inhibited by EDTA and contain at least one zinc atom at the active site that facilitates the hydrolysis of the beta-lactam ring (Frere et al., 2005). Serine enzymes (contain serine at the active site) emerged in the mid to late 1980s and were found to be inhibited by clavulanic acid and tazobactam but not by EDTA (Medeiros and Hare, 1986; Yang et al., 1990, Rasmussen et al., 1996).

1.3.5.1 The Ambler Molecular Classification

The Ambler Molecular Classification (Fig: 8) is based on amino acid homology and not phenotypic characteristics and resulted in four known classes of beta lactamases (Huovinen et al., 1988; Jaurin and Grundstrom, 1981; Ambler, 1980). Carbapenemases are divided into three of four classifications which include classes A, B, and D. Classes A and D which include serine at their active site are considered as serine carbapenemases, where as class B contains metallo carbapenemases with zinc in its active site (Ambler, 1980, Paterson and Bonomo, 2005). Class B metallo carbapenemases have a wide spectrum activity against carbapenems, penicillins, and extended spectrum cephalosporins but not aztreonam (Queenan and Bush, 2007; Patel et al., 2009). These enzymes can be found in Gram negative bacteria such as Enterobacteriacea and others (Walsh et al. 2005).

The major family of class A serine carbapenemases include KPC enzymes (Ambler et al., 1991). To date, eight families of acquired metallo beta-lactamases, including IMPs, VIMs, SPM-1, GIM-1, AIM-1, SIM-1, NDM-1 and DIM-1, have been identified (Zhao and Hu, 2010; Lee et al., 2005; Yong et al., 2009; Poirel et al., 2010). IMPs and VIMs, which belong
to the subclass B1, are the most frequent metallo beta-lactamases acquired by Gram-negative bacilli (Zhao and Hu, 2010; Walsh et al., 2005). In class B metallo carbapenemases the mainly three types refers to: IMP (imipenemase), VIM (Verona integron-encoded metallo-beta-lactamase) and the recently reported NDM-1 (New Delhi metallo-beta-lactamase-1) (Ambler, 1980; Moellering, 2010). Class D include OXA type carbapenemase (oxacillin-hydrolyzing) (Naas and Nordmann, 1999).

Figure 8: The Ambler Molecular Classification for carbapenemases

1.4 Carbapenemases Point Mutation Genes

1.4.1.1 KPC-type Carbapenemase (Class A)

The KPC-type carbapenemases refers to *Klebsiella pneumoniae* carbapenemases. They are the most commonly occurring molecular class A and functional group 2f. KPCs can hydrolyze multiple different antimicrobial agents such as fluoroquinolones, aminoglycosides, and β-lactams including carbapenems, cephalosporins, penicillins, and aztreonam (Endimiani et al.,
2009; Castanheira et al., 2008) and are inhibited by clavulanic acid and tazobactam (Queenan and Bush, 2007).

To date, ten KPC gene variants have been reported and classified from KPC-2 to KPC-11 while KPC-1 was found to be identical to KPC-2 (Yigit et al. 2008). The KPC-2 to KPC-11 genes are characterized by nonsynonymous single nucleotide substitutions within four codons (nucleotides: 147, 308, 716, and 814) (Wolter et al. 2009).

Multiple studies reported KPC genes worldwide; KPC-2 (Nordmann et al., 2009), KPC-3 (Nordmann et al., 2009), KPC-4 (Livermore et al. 2008; Palepou et al. 2005, Robledo et al. 2007; Robledo et al. 2010), KPC-5 (45), KPC-6 (Aquino et al., 2008), KPC-7 (Perez et al. 2010), KPC-8 (Gregory et al. 2010), and KPC-10 (Robledo et al. 2010) have been described in numerous countries, with KPC-2 and KPC-3 accounting for most epidemic outbreaks (Nordmann et al., 2009).

The KPCs are predominantly found in K. pneumoniae; however, they have also been found in many other Enterobacteriaceae including Escherichia coli (Carl et al., 2008) Enterobacter species (Bratu (A) et al., 2005) Salmonella species (Miriagou (A) et al., 2003) Proteus mirabilis (Tibbetts et al., 2008) and Citrobacter freundii (Marı et al., 2010) as well as many other non Enterobacteriaceae including Pseudomonas aeruginosa (Villegas et.al., 2007) and most recently in an Acinetobacter baumannii strain (Robledo et.al., 2010).

The first KPC isolates Klebsiella pneumoniae was found in the United States in North Carolina in 2001 and then spread in New York (Yigit et al., 2001; Patel et al, 2009; Kitchel et al., 2009). However, KPCs are now widely distributed worldwide and multiple studies reported the presence of KPC such as in Israel, China, Greece, South America, Germany and India (Queenan and Bush, 2007; Patel et al, 2009, Yigit et al., 2001, Arnold et al., 2011; Grundmann et al., 2010; Steinmann et al., 2011).

In 2008, KPC-2 producing Klebsiella pneumoniae outbreak was reported in southern part of Germany (Wendt et al., 2010). In 2005, Naas and his colleagues reported the first case of
KPC-producing *Klebsiella pneumoniae* in France while the first outbreak of KPC-producing *Klebsiella pneumoniae* outside the United States was in Israel (Leavitt et al., 2007).

Infections produced by KPCs are frequently identified among nosocomial pathogens and are associated with treatment failure and high mortality rates that reached at least 50% (Landman et al., 2007; Bratu (B) et al., 2005; Gasink et al., 2009). Combination therapy is recommended for the treatment of KPC infections (Lee and Burgess, 2012). The multiplex real-time PCR assay using molecular beacons was found to be robust, sensitive, and specific, allowing for high-throughput detection and classification of all KPC variants (Chen et al., 2011).

### 1.4.1.2 IMP-type Carbapenemase (Class B)

IMP-type beta lactamase refers to imipenemase, the Ambler Molecular class B (metallo carbapenemases) (Ambler, 1980), group 3 in the Bush-Jacoby Functional classifications (Bush et al., 1995). These enzymes hydrolyze carbapenems and extended-spectrum beta-lactams (Livermore and Woodford, 2000) but are not inhibited by beta lactamase inhibitors such as as clavulanic acid, sulbactam, and tazobactam (Bush, 2001).

The first identified acquired metallo beta-lactamases was IMP-1. It was detected in a clinical isolate of *S. marcescens* from Japan in 1994 (Osano et al., 1994; Ito et al., 1995). Until the year of 2008, 24 variants of the IMP have been determined (Lahey Clinic, 2008). Recent investigations showed that the IMP family has at least 33 unique IMP variants (http://www.lahey.org/Studies), which may differ widely in regard to the primary sequence and biochemical activity. In August 2012, IMP-28 isolate was discovered in *Klebsiella oxytoca* in Madrid, Spain (Pérez-Llarena et al., 2012). Several IMP variants which are close to IMP-1 have been reported in the last few years; IMP-2 from an isolate of *A. baumannii* in Italy (Riccio et al., 2000), IMP-3 from *Shigella flexneri* in Japan (Iyobe et al., 2000), and IMP-4 from *Acinetobacter* and *Citrobacter* spp in China (Chu et al., 2001).
The IMP-7 shares 91% amino acid identity with IMP-1 (Gibb et al., 2002), while the IMP-10 is a point mutation derivative of IMP-1 with a single base replacement leading to amino acid alteration (Iyobe et al., 2002).

The \textit{bla}IMP genes are usually horizontally transferred by integrons (Class one) carried by transferable large plasmids (Arakawa et al., 1995; Poirel et al., 2000, Shibata et al., 2003). IMP was found to be among \textit{Enterobacteriacea}, \textit{Pseudomonas aeruginosa}, \textit{Acinetobacter baumannii} and other bacteria in Japan (predominantly IMP-1) as well as in Europe and the Far East (Hirakata et al., 1998; Ito et al., 1995; Senda (A) et al., 1996; Senda (B) et al., 1996; Riccio et al., 2000; Hawkey et al., 2001; Yano et al., 2001).

### 1.4.1.3 VIM-type Carbapenemase (Class B)

VIM-type beta lactamase refers to Verona Integron-encoded Metallo-\(\beta\)-lactamase. As IMP, it is in the Ambler Molecular class B (metallo carbapenemases) (Ambler, 1980), group 3 in the Bush-Jacoby Functional classifications (Bush et al., 1995). Until 2008, 22 VIM variants have been determined (Lahey Clinic, 2008). The name Verona Integron-encoded Metallo-\(\beta\)-lactamase was given because the VIM was first isolated in Verona, Italy, in 1997 (Lauretti et al., 1999).

These enzymes hydrolyze carbapenems and extended-spectrum beta-lactams (Livermore and Woodford, 2000) but are not inhibited by beta lactamase inhibitors such as as clavulanic acid, sulbactam, and tazobactam (Bush, 2001).

VIM-2 is considered the most reported metallo beta-lactamase worldwide (Walsh et al., 2005).

Carbapenem resistance related VIM beta-lactamase production is considered a serious problem since they have high ability to spread (Richet et al., 2001). The VIM gene as IMP gene is horizontally transferable.
A total of 27 VIM family variants (VIM-1–27) have been assigned in the Lahey Clinic (www.lahey.org/Studies) and were found mainly in *Pseudomonas aeruginosa* (Castanheira et al., 2009; Siarkou et al., 2009; Schneider et al., 2008) and other pathogens such as *Pseudomonas putida* (Lombardi et al., 2002), *Escherichia coli* (Miriagou (B) et al., 2003), *Klebsiella pneumoniae* (Giakkoupi (A) et al., 2003), *Klebsiella oxytoca* (Kristóf et al., 2010), *Enterobacter cloacae* (Lee et al., 2008), *Acinetobacter baumannii* (Tsakris et al., 2006), *Proteus mirabilis* (Vourli et al., 2006), *Citrobacter freundii* (Yan et al., 2002) and *Serratia marcescens* (Lee et al., 2008).

Treatment options of VIM-producing organisms are considered limited. Multiple studies showed that combination therapy increase the patient survival and is considered more effective against infections caused by *P. aeruginosa* (Chamot et al., 2003; Micek et al., 2005; Parkins et al., 2007). In a study carried out by Parkins and his colleagues in the period of 2002 and 2004 suggested piperacillin–tazobactam as the backbone of most of the treatment regimens, but was still associated with frequent treatment failure. Furthermore, they commonly used aztreonam as a second beta lactam agent (Parkins et al., 2007), despite animal data showing questionable efficacy (Bellais et al., 2002).

### 1.4.1.4 OXA-48-type Carbapenemase (Class D)

OXA-48 refers to Oxacillinase beta lactamase, the Ambler Molecular Class D (Ambler, 1980), and group 2d according to Bush-Jacoby Classification (Bush et al., 1995). OXA represented one of the most prevalent plasmid-encoded beta-lactamase families in the late 1970s and early 1980s (Matthew, 1979; Medeiros, 1984; Simpson et al., 1980). They have been found mainly in *Enterobacteriaceae* and *P. aeruginosa* (Bush and Sykes, 1987; Naas and Nordmann, 1999). OXA-48 was found in *E. coli* as well as in *Enterobacter cloacae* (Dimou et al., 2012) and others. The First OXA beta lactamase with carbapenemase activity was described by Paton et al. in 1993 in an *Acinetobacter baumannii* isolate, which is
found to be the most prevalent organism that carries the OXA carbapenemases (Afzal-Shah and Livermore, 1998).

OXA-48 is plasmid encoded gene and have less than 50% amino acid identity to the other OXA members. It spread into multiple strains of several Enterobacteriaceae species by horizontal transfer (Dimou et al., 2012). It was discovered in a clinical K. pneumoniae isolate from Istanbul, Turkey (Poirel et al., 2004). OXA-48 has the ability to hydrolyze penicillins, imipenem, and expanded-spectrum cephalosporins (Poirel et al., 2004). In a study carried out by Mimoz and his colleagues, a lethal peritonitis model was induced in mice with a Klebsiella pneumoniae isolate producing the carbapenemase OXA-48. A single dose (up to 100 mg/kg) of the antibiotic piperacillin-tazobactam, imipenem-cilastatin, ertapenem, or cefotaxime has been injected. Ceftazidime had the highest efficacy in vivo. Therefore, they recommended ceftazidime for the treatment of infections caused by OXA-48 producers if they do not coproduce an extended-spectrum beta-lactamase or a plasmid-mediated AmpC cephalosporinase (Mimoz et al., 2012).

### 1.4.1.5 NDM-1-type Carbaenemase (Class B)

NDM-1 type refers to New-Delhi Metallo beta-lactamase, the Ambler Molecular class B and group 3 according to Bush-Jacoby functional classification. It is a broad-spectrum β-lactamase (carbapenemase) that is able to inactivate all β-lactams except aztreonam (Yong et al., 2009). The name New Delhi came from the Indian origin of the first reported case which was in 2009 in a Swedish patient who travelled to New Delhi and acquired a UTI caused by carbapenem resistant Klebsiella pneumoniae and E. coli strains. These strains showed multi resistance to all antimicrobial agents except colistin (Yong et al., 2009). Investigations for this patient indicated an NDM-1 positive E. coli. The NDM-1 gene can be easily transferred due to its location on plasmids which are easily transferable (Yong et al., 2009). These plasmids also carry a number of other genes which
convey resistance to all aminoglycosides, macrolides, and sulfamethoxazole. Consequently, making these isolates multidrug resistance and in some cases to all antibiotics including tigecycline and colistin (Kumarasamy et al., 2010). NDM-1 convey’s bacteria cause a wide range of infections, both in the community as well as in hospital settings (Kumarasamy et al., 2010; Roy et al., 2011; Khan and Nordmann, 2012).

NDM-1 was found to be among different Enterobacteriaceae spp including Klebsiella pneumonia, E. coli, E. cloacae (Sonnevend et al., 2013; Kumarasamy et al., 2010), Proteus spp (Kumarasamy et al., 2010), Citrobacter freundii (Poirel et al., 2011; Sonnevend et al., 2013; Kumarasamy et al., 2010), K. oxytoca (Kumarasamy et al., 2010), Providencia spp (Kus et al., 2011), M. Morganii (Kus et al., 2011), Shigella boydii, and Vibrio cholerae (Walsh et al., 2011).

Since the treatment options for infections caused by NDM-1 producing bacteria are very limited, the NDM-1 producing organisms are named the world’s newest “superbugs”. The term “superbugs” is a bacterium that carries resistance genes for many antibiotics (Salcido, 2010).

The best treatments for an infection caused by bacteria that produce NDM-1 are colistin and tigecycline (Stone et al., 2011). Colistin is infrequently used because of its toxicity, but it is often the only antibiotic to which NDM-1-producing bacteria are susceptible to (Falagas and Kasiakou, 2006). Few NDM-1-producing bacteria have shown sensitivity aztreonam (Liang et al., 2011).

1.4.2 QRDR Point Mutation Genes

1.4.2.1 QnrA-, QnrB-, and QnrS-types

Resistance to quinolones is classically chromosomally mediated. However, a family of plasmid-encoded quinolone resistance genes (qnrA, qnrB, qnrC and qnrS) has recently been described (Robicsek (B) et al., 2006). The qnrA found to have at least 6 variants (qnrA1-
qnrA6) as well as qnrB 6 variants (qnrB1-qnrB6) while qnrS 2 variants (qnrS1-qnrS2). The qnrB and qnrS share 41% and 60% amino acid identity with qnrA (Shin et al., 2009; Nordmann and Poirel, 2005). They act by protecting both DNA gyrase and topoisomerase IV from quinolone inhibition (Yamane et al., 2007).

Qnr variants have been observed in clinical isolates, more frequently among strains producing plasmid-mediated, extended-spectrum β-lactamases (Robicsek (B) et al., 2006).

Three major groups of Qnr determinants, qnrA, qnrB, and qnrS, have been identified in various enterobacterial species such as Klebsiella pneumoniae (Chen el al., 2006), Salmonella enterica (Cheung et al., 2005), Shigella flexneri (Hata et al., 2005), Escherichia coli (Mammeri et al., 2005), Enterobacter cloacae (Paauw et al., 2006), and many others.

The first plasmid-mediated quinolone resistance Qnr (later termed qnrA) was from the United States and reported in 1998 for a Klebsiella pneumoniae isolate (Martínez- Martínez et al., 1998). According to CLSI guidelines, Qnr provides resistance to nalidixic acid but only reduced susceptibility to fluoroquinolones.

The treatment of quinolone resistant producing organism infections is considered limited. Ciprofloxacin, a member of the large and widely used fluoroquinolone group of antimicrobial drugs, is considered the empirical choice treatment of infections in adults (Jacoby et al. 2006).

### 1.4.2.2 AAC-6-Ib-type

AAC-6-Ib-type refers to a new variant of the common aminoglycoside acetyltransferase (Melano et al., 2003; Park et al., 2006). This gene harbours two base pair substitutions that are capable of acetylating some of the fluoroquinolones such as ciprofloxacin and norfloxacin and aminoglycosides, thereby reducing their activities (Robicsek (C) et al., 2006). Previous studies have described several mechanisms for aminoglycoside resistance including the bacterial expression of drug-metabolizing enzymes, such as aminoglycoside N-6′-
acetyltransferase-Ib (aac(6')-Ib) being the most common mechanism of resistance to aminoglycoside antibiotics, especially in Gram-negative clinical isolates (O'Neill, 2008). The aac(6')-I is classified into at least six genes (aac(6')-Ia to aac(6')-If (Galimand et al., 1993). The aac-6-Ib gene have been identified in various enterobacterial species such as Enterobacter spp. (Kim et al., 2009), Escherichia coli (Frsson et al., 2011; Kim et al., 2009; Park et al., 2006), Klebsiella pneumoniae (Kim et al., 2009), Pseudomonas aeruginosa (Galimand et al., 1993). Acinetobacter spp. (Miller et al., 1985; Teran et al., 1991), Serratia marcescens (Lambert et al., 1994), Enterobacter cloacae (Kim et al., 2010), and many others. The aac(6')-Ib gene is considered the most prevalent aminoglycoside modifying enzyme that confers resistance to tobramycin, kanamycin, and amikacin. It was first identified in 1986 in Klebsiella pneumoniae isolates (Tolmasky et al., 1986).

Although the treatment options of aac(6')-Ib producing organisms infections are very limited, the EUCAST reported amikacin as intermediate for Enterobacteriaceae which are intermediate or resistant to tobramycin and susceptible to gentamicin and amikacin.

1.5 Detection of Carbapenemases and QRDR Genes

Detection of resistance genes is a crucial infection control issue because:

1. They are often associated with extensive, sometimes total, antibiotic resistance
2. Resistant organisms that have acquired a carbapenemase can be vectors responsible for carbapenemase transmission to other organisms (Thomson, 2010).

Detection of carbapenemases is difficult. It can be detected by phenotypic as well as genotypic methods (Tenover, 2006). Carbapenemase producers are screened first by susceptibility testing The EUCAST recommended procedure to detect carbapenemases producing organisms involves an initial disk diffusion screening with disks containing imipenem, meropenem or ertapenem. Meropenem is considered the best for sensitivity and specificity in terms of detecting carbapenemase-producers (Nordmann et al., 2012, Vading et
al., 2011). On the other hand, ertapenem has excellent sensitivity, but poor specificity (relative instability to ESBLs and AmpC β-lactamases in combination with porin loss) (Nordmann et al., 2012). Carbapenemase-producing Enterobacteriaceae often have MICs below the clinical carbapenem breakpoints (Nordmann et al., 2012; EUCAST, 2012; Tato et al., 2007). The MICs of carbapenemase-producing strains differ between strains, and some strains have MICs for carbapenems below the current clinical susceptibility breakpoints (Nordmann and Nass, 2009; Miriagou et al., 2010).

Carbapenemases identification based on phenotype-techniques such as the modified Hodge test, are considered not highly sensitive and specific (Nordmann et al., 2012; Pasteran et al., 2010). This is a phenotypic test which could be used to determine if reduced susceptibility to carbapenems is mediated by a carbapenemase (Anderson et al., 2007). On the other hand, Amjad A and his colleagues concluded that modified Hodge test is a simple test that can be performed in the routine laboratory (Amjad et al., 2011). The test showed 100% sensitivity and specificity for detection of KPCs according to Lee and his colleagues (Lee et al., 2001). In 2012, Nordmann and his colleagues developed the Carba NP test to rapidly identify carbapenemase producers in Enterobacteriaceae. The test based on in vitro hydrolysis of a carbapenem, imipenem (Fig: 9). Results showed that this test was 100% sensitive and specific compared with molecular-based techniques. Furthermore, it was found to be rapid (<2 hours), inexpensive technique that may be implemented in any laboratory (Nordmann (B) et al., 2012).

For the detection of fluoroquinolone resistance producing organisms, susceptibility testing with disks containing ciprofloxacin, norfloxacin, and nalidixic acid (which is a marker for target mutations) should be performed according to EUCAST (Cavaco and Aarestrup, 2009).
Figure 9: Carba NP test for identification of carbapenemases in *Enterobacteriaceae*

Multiple studies detected the resistance genes via multiplex PCR (Chmelnitsky et al., 2008; Moquet et al., 2011; Shibata et al., 2003). Molecular detection of carbapenemase genes is widely used but it is considered expensive, time consuming (at least 12–24 hours), and requires substantial expertise (Nordmann (B) et al., 2012).
2 Research Objectives:

The general objectives of this research are:

1. Genotype carbapenemases-producing organism isolates to determine the most common carbapenemases genes at the University Hospital of Leipzig.
2. Determine genotypic-phenotypic relationships of these isolates.
3. Determine the Quinolone Resistance Determining Region (QRDR) mutations.
4. Determine alterations of resistance mechanisms over time in the KPC-positive organisms.
3  Materials and Methods

3.1  Materials

3.1.1  Bacteria

4.1.1.1 Clinical Isolates

A total of 50 clinical isolates of KPC-producing organisms were collected from hospitalized patients admitted to the University Hospital of Leipzig. Duplicate isolates were excluded, so that one isolate was collected for a patient, and those isolates were collected from a variety of clinical specimens including; Urine, blood, wounds, and others.

4.1.1.2 Bacterial Strains

Strains meeting the study criteria which previously confirmed as a KPC producing organism were stored at -70°C. Consequently, all these strains were subcultured and used in the molecular identification process.

4.1.1.3 KPC Genes Control Strains

KPC 375/08 K. pneumonia strain was obtained from the University hospital of Leipzig/Institut of Microbiology and infection epidemiology and was used as a positive control strain for the KPC gene as well as K. pneumonia (Kpn1056) and E.coli (Eco3171) strains that were obtained from Freiburg University. Citrobacter freundii (Cit37, -38, -39, -43, and -44) and Enterobacter spp (Ent458) strains were obtained from Freiburg University and used as a positive control for qnrA, qnrB, and qnrS genes. P. aeruginosa VIM-2 strain was obtained from the University Hospital of Leipzig/Institut of Microbiology and infection epidemiology and used as a positive control strain for the VIM gene. Acinetobacter boumani IMP-1 strain was obtained from the University Hospital of Leipzig/Institut of Microbiology and infection epidemiology and used as a positive control for the IMP gene. E.coli (strain
396/11) was obtained from Robert Koch-Institut and used as a positive control for OXA-48 gene. *K. pneumoniae* (strain 10-O 3/11) was obtained from Robert Koch Institut and used as a positive control for NDM-1 gene. *K. pneumoniae* (strain GNS-4240) was obtained from Ruhr University of Bochum and used as a positive control for aac-6’-Ib gene.

### 3.1.2 Antimicrobial Susceptibility Testing and Culture Media

Potential KPC-producing strains were previously confirmed at the Institute for Microbiology of the University Hospital of Leipzig by MIC values according to EUCAST guideline.

MacConkey agar was used for the KPC-producing isolates subculture.

### 3.1.3 Electrophoresis Chemicals

Agarose, ethidium bromide, loading die, and PCR ladder (100bp) were prepared for running the study genes.

### 3.1.4 PCR Primers and Chemicals

All strains were examined for the presence of KPC, qnrA, qnrB, qnrS, aac(6’)-Ib, IMP, VIM, OXA-48, and NDM-1 genes using multiplex PCR. The primers, their sequences and amplicon size which were used in this study are shown in table 1.

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Sequence (5’-3’ direction)</th>
<th>Amplicon size in bp*</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>bla-KPC-F</td>
<td>5’-ATGTCACTGTATCGCCGTCT-3’</td>
<td>424bp</td>
<td>(Pillai et al., 2009)</td>
</tr>
<tr>
<td>bla-KPC_R</td>
<td>5’-TTTTCAGAGCCTTACTGCCC-3’</td>
<td>424bp</td>
<td>(Pillai et al., 2009)</td>
</tr>
<tr>
<td>qnr-A-F</td>
<td>5’-TTTCTCACGCCAGGGATTG-3’</td>
<td>516bp</td>
<td>(Robicsek (C) et al., 2006)</td>
</tr>
<tr>
<td>qnr-A-R</td>
<td>5’-GATCGGCAAAGGTAGTCA-3’</td>
<td>516bp</td>
<td>(Robicsek (C) et al., 2006)</td>
</tr>
<tr>
<td>qnr-B-F</td>
<td>5’-GATCGTGAAAGCAGGAGG-3’</td>
<td>469bp</td>
<td>(Robicsek (C) et al., 2006)</td>
</tr>
</tbody>
</table>
Table 1: Nucleotide sequence, and size of the primers used for multiplex PCR

<table>
<thead>
<tr>
<th>Primer</th>
<th>Sequence</th>
<th>Size (bp)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>qnr-B-R</td>
<td>5'-ACGATGCCTGGTAGTTGTCC-3'</td>
<td>469bp</td>
<td>(Robicsek (C) et al., 2006)</td>
</tr>
<tr>
<td>qnr-S-F</td>
<td>5'-ACGACATTCGTCACACTGCAA-3'</td>
<td>417bp</td>
<td>(Robicsek (C) et al., 2006)</td>
</tr>
<tr>
<td>qnr-S-R</td>
<td>5'-TAAATGGCACCCTGTAGGC-3'</td>
<td>417bp</td>
<td>(Robicsek (C) et al., 2006)</td>
</tr>
<tr>
<td>IMP-F</td>
<td>5'-GGAATAAGAGTGGCTTAAYTC-3'</td>
<td>447bp</td>
<td>*</td>
</tr>
<tr>
<td>IMP-R</td>
<td>5'-TCGTTTAAYAAAAACACCACC-3'</td>
<td>447bp</td>
<td>*</td>
</tr>
<tr>
<td>VIM-F</td>
<td>5'-GATGGTGTGGTGGTCGATA-3'</td>
<td>390bp</td>
<td>*</td>
</tr>
<tr>
<td>VIM-R</td>
<td>5'-CGAATGCGCGACGACCAG-3'</td>
<td>390bp</td>
<td>*</td>
</tr>
<tr>
<td>OXA-48-F</td>
<td>5'-GCGTGGTTAAGGATGAACAC-3'</td>
<td>438bp</td>
<td>(Poirel (2) et al., 2011)</td>
</tr>
<tr>
<td>OXA-48-R</td>
<td>5'-CATCAAGTTCAAACCAACCAG-3'</td>
<td>438bp</td>
<td>(Poirel (2) et al., 2011)</td>
</tr>
<tr>
<td>NDM-1-F</td>
<td>5'-GGTTTGCGATCTGGTTTTC-3'</td>
<td>621bp</td>
<td>(Poirel (2) et al., 2011)</td>
</tr>
<tr>
<td>NDM-1-R</td>
<td>5'-CGGAATGGCTCATCAGCATC-3'</td>
<td>621bp</td>
<td>(Poirel (2) et al., 2011)</td>
</tr>
<tr>
<td>aac(6')-Ib-F</td>
<td>5'-TTGCGATGCTCTTATGAGTGCTA-3'</td>
<td>482bp</td>
<td>(Chemelnitsky et al., 2009)</td>
</tr>
<tr>
<td>aac(6')-Ib-R</td>
<td>5'-CTCGAATGCCTGGCGTGGTTT-3'</td>
<td>482bp</td>
<td>(Chemelnitsky et al., 2009)</td>
</tr>
</tbody>
</table>

* Leipzig University Hospital/Institut of Microbiology and infection epidemiology

3.1.5 Pulsed Field Gel Electrophoresis (PFGE)

All PCR products were examined using PFGE. The PFGE allows separating much larger pieces of DNA than conventional agarose gel electrophoresis (Achtman and Morelli, 2001). The performance of PFGE is similar to a standard gel electrophoresis except that instead of constantly running the voltage in one direction, the voltage in PFGE is periodically switched among three directions. In addition, this technique easily measures the genome size and characterizes various strains at the DNA level (Basim and Basim, 2001).
3.2 Methods

3.2.1 Carbapenemases Clinical Isolates

All clinical isolates which were collected from patients of the University Hospital of Leipzig as KPC producing organisms were stored at -70°C. Isolates were subcultured by streaking on MacConkey agar and incubating them at 37°C for 24 hours and their presumptive identification was made on the basis of growth characteristics and a set of biochemical tests.

3.2.2 Disc Diffusion Method and MIC Values

The procedure was carried out according to EUCAST guidelines. The antimicrobial disks which were used contain imipenem, ertapenem, tigecycline, and other antibiotics. Results were interpreted and recorded as resistance (R), Intermediate (I), or susceptible (S). The isolates antimicrobial susceptibility test and the MIC values results were taken from the Institute for Microbiology of the University Hospital of Leipzig.

3.2.3 Pulsed Field Gel Electrophoresis

All PCR products were examined using PFGE according to BIO-RAD CHEF genomic DNA plug Kit manual as described below.

Bacterial culture was inoculated into 50 ml of LB Broth and then a final concentration of 180µg/ml of chloramphenicol were added and incubated for 1 hour. A twenty-fold dilution of the bacterial suspension was made using 1 ml bacteria, 1 ml Gram Crystal Violet, and 18 ml saline. The 2% CleanCut agarose solution was melt using a microwave and the solution was equilibrated to 50°C in a water bath. For a final concentration of 1% agarose, 0.5 ml of Cell Suspension Buffer was used per ml of agarose plugs. For each ml of agarose plugs, 5 x 10^8 cells were removed then centrifuged for 3 minutes in a microcentrifuge at 10000 rpm at 4°C. The cells were resuspended in the cell suspension buffer and the cell suspension was equilibrated to 50°C. The calculated volume of 2% CleanCut agarose was combined with the
cell suspension and they were mixed gently. The cell/agarose mixture was kept at 50°C then the mixture was transferred to plug molds using sterile transfer pipettes and then the agarose was allowed to solidify. The solidified agarose plugs were pushed into a conical centrifuge tube containing lysozyme solution the plugs were incubated for 2 hours at 37°C. The lysozyme solution was then removed and the plugs were rinsed with sterile water. Proteinase K was then added as a reaction buffer for each ml of agarose plugs and then the plugs were incubated overnight at 50°C without agitation. The plugs were then washed four times in 1x Wash Buffer and were incubated for 1 hour at room temperature with gentle agitation. Finally, the plugs were stored at 4°C in 1x Wash Buffer.

3.2.4 Detection of the KPC Gene

Multiplex PCR was utilized to detect the presence of KPC gene. The DNA extraction was performed as described below.

3.2.4.1 DNA Extraction

The extraction of the isolates DNA was made according to the manual of Qiagen. Few bacterial colonies were harvested in 1ml normal saline and mixed by vortexing until dissolved completely. The suspension was centrifuged for 5 minutes at 7500 round/m. The supernatant was removed and the pellet was then resuspended in 180µl of ALT buffer and then was mixed by vortexing until it is completely resuspended. A volume of 20µl of Proteniase was added to the solution and mixed using the vortex. The suspension was left in the shaker for 1 hour at 56°C. The solution was then mixed by the vortex for 15 seconds and centrifuged for 1 min at 8000 round/min. The AL lyses buffer was added at a volume of 200µl and the tubes were mixed by vortex and centrifuged for 20 seconds at 8000 round/min. A volume of 200µl of Ethanol was added to the suspension as a neutralization solution then mixed by vortex and centrifuged for 1 min at 8000 round/min. The supernatant was removed and then placed in another eppendorf tube with a spin column, then centrifuged for 1 min at 8000 round/min.
The spin column was then transferred to a new eppendorf tube. The first washing solution (AW1) with a volume of 500µl was added and the suspension was then centrifuged for 1 min at 8000 round/min. The spin column was then transferred to a new eppendorf tube. A volume of 500µl of the second washing solution (AW2) was added and centrifuged for 3 min at 14000 round/min. The spin column was transferred to a new eppendorf tube and centrifuged for 1 min at 14000 round/min as an additional step to make sure that the washing solution is completely gone. The spin column was then placed in a new eppendorf tube and 130µl of AE buffer was added. The suspension was incubated for 1 min at room temperature and then centrifuged for 1 min at 8000 round/min. Finally, the spin column was removed and the suspension formed which contains the DNA was kept at -70°C until used for all of the research genes.

3.2.4.2 The Master Mix and the Working Solution

The Master Mix reagents were used in concentrations which gave the best gene bands in the gel electrophoresis. The working solution of the PCR master mix was prepared by adding 0.2µl of each primer (the forward and reverse primers), 5µl of 10Xbuffer, 4µl of 25mM MgCl₂, 1,25µl dNTP’s 10mM, and 0.25µl of Taq polymerase and Finally 37.1µl of the free Dnase/Rnase water. Each PCR reaction was carried out using 2µl DNA and 48µl of the previously prepared Master Mix in a final volume of 50µl.

3.2.4.3 The PCR Conditions

The amplification conditions were used according to previously programmed PCR conditions made by the microbiology and infections epidemiology laboratory staff and involved an initial denaturation step at 95°C for 2 min, 30 cycle denaturation at 95°C for 20 seconds, annealing at 55°C for 30 seconds, extension at 72°C for 40 seconds, followed by a final extension step at 72°C for 3 min and then cooled at 4°C for ∞. The PCR annealing
temperature was considered according to the following equation: \[4 \times (C+G) + 2 \times (A+T) - 5^\circ C.\]

The PCR products were detected in 1.5% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed. Precautions were taken to prevent the samples from being contaminated by each other or from the skin and to keep out of the toxicity of the Ethedium Bromide. These precautions included the use of pre aliquoted PCR reagents, gloves, and disposable tips. The preparation of the amplification reaction mixtures and the analysis of the amplified products were performed in separate areas.

3.2.5 Detection of qnrA, qnrB Genes

Multiplex PCR was utilized to detect the presence of qnrA and qnrB genes separated. The DNA extraction was performed as described above.

3.2.5.1 The Master Mix and the Working Solution

The working solution of the PCR master mix was prepared by adding 2\(\mu\)l of each primer (the forward and reverse primers), 5\(\mu\)l of 10Xbuffer, 5\(\mu\)l of 25mM MgCl\(_2\), 2\(\mu\)l of 10mM dNTP’s, and 0.5\(\mu\)l of Taq polymerase and Finally 29.5\(\mu\)l of the free Dnase/Rnase water. Each PCR reaction was carried out using 4\(\mu\)l DNA and 46\(\mu\)l of the previously prepared Master Mix in a final volume of 50\(\mu\)l. This Master Mix was used at the same reagents concentrations for both qnrA and qnrB genes.

3.2.5.2 The PCR Conditions

The amplification conditions were the same in detection the qnrA and the qnrB genes and were used according to Robicsek (C) et al., 2006 with few modifications. They involved an initial denaturation step at 94\(^\circ\)C for 1 min, 32 cycle denaturation at 94\(^\circ\)C for 20 seconds, annealing at 53\(^\circ\)C for 30 seconds, extension at 72\(^\circ\)C for 40 seconds, that is followed by a final
extension step at 72°C for 6 min and then cooled at 4°C for ∞. The PCR annealing temperature was considered according to the following equation: 4 * (C+G) + 2 * (A+T) - 5°C.

The PCR products were detected in 1.5% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.

3.2.6 Detection of qnrS Gene

Multiplex PCR was utilized to detect the presence of qnrS gene. The DNA extraction was performed as described above.

3.2.6.1 The Master Mix and the Working Solution

The working solution of the PCR master mix was prepared by adding 4µl of each primer (the forward and reverse primers), 5µl of 10Xbuffer, 5µl of 25mM MgCl2, 2µl of 10mM dNTP`s, and 0.5µl of Taq polymerase and Finally 25.5µl of the free Dnase/Rnase water. Each PCR reaction was carried out using 4µl DNA and 46µl of the previously prepared Master Mix in a final volume of 50µl.

3.2.6.2 The PCR Conditions

The amplification conditions were used according to Robicsek (C) et al., 2006 with few modifications and involved an initial denaturation step at 94°C for 1 min, 32 cycle denaturation at 94°C for 20 seconds, annealing at 53°C for 30 seconds, extension at 72°C for 40 seconds, that is followed by a final extension step at 72°C for 6 min and then cooled at 4°C for ∞. The PCR annealing temperature was considered according to the following equation: 4 * (C+G) + 2 * (A+T) - 5°C.
The PCR products were detected in 1.5% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.

### 3.2.7 Detection of IMP Gene

Multiplex PCR was utilized to detect the presence of IMP gene. The DNA extraction was performed as described above.

#### 3.2.7.1 The Master Mix and the Working Solution

The working solution of the PCR master mix was prepared by adding 0.4µl of each primer (the forward and reverse primers), 5µl of 10Xbuffer, 4µl of 25mM MgCl₂, 1.25µl of 10mM dNTP’s, and 0.25µl of Taq polymerase and Finally 34.7µl of the free Dnase/Rnase water. Each PCR reaction was carried out using 4µl DNA and 46µl of the previously prepared Master Mix in a final volume of 50µl.

#### 3.2.7.2 The PCR Conditions

The amplification conditions were used according to previously programmed PCR conditions made by the microbiology and infections epidemiology laboratory staff and involved an initial denaturation step at 95°C for 2 min, 30 cycle denaturation at 95°C for 1 min, annealing at 53°C for 1 min, extension at 72°C for 1 min, that is followed by a final extension step at 72°C for 3 min and then cooled at 4°C for ∞. The PCR annealing temperature was considered according to the following equation: 4 * (C+G) + 2* (A+T) - 5°C.

The PCR products were detected in 1.5% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.
3.2.8 Detection of VIM and OXA-48 Genes

Multiplex PCR was utilized to detect the presence of VIM and OXA-48 genes separated. The DNA extraction was performed as described above.

3.2.8.1 The Master Mix and the Working Solution

The working solution of the PCR master mix was prepared by adding 0.3µl of each primer (the forward and reverse primers for VIM and OXA-48 genes), 5µl of 10Xbuffer, 4µl of 25mM MgCl₂, 1.25µl of 10mM dNTP’s, and 0.25µl of Taq polymerase and Finally 34.3µl of the free Dnase/Rnase water. Each PCR reaction was carried out using 4µl DNA and 46µl of the previously prepared Master Mix in a final volume of 50µl.

3.2.8.2 The PCR Conditions

The amplification conditions were used according to previously programmed PCR conditions made by the microbiology and infections epidemiology laboratory staff and involved an initial denaturation step at 94°C for 10 min, 36 cycle denaturation at 94°C for 30 seconds, annealing at 52°C for 40 seconds, extension at 72°C for 50 seconds, that is followed by a final extension step at 72°C for 5 min and then cooled at 4°C for ∞. The PCR annealing temperature was considered according to the following equation: 4 * (C+G) + 2* (A+T) - 5°C.

The PCR products were detected in 1.5% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.

3.2.9 Detection of NDM-1 Gene

Multiplex PCR was utilized to detect the presence of NDM-1 gene. The DNA extraction was performed as described above.
3.2.9.1 The Master Mix and the Working Solution

The working solution of the PCR master mix was prepared by adding 0.5µl of each primer (the forward and reverse primers), 5µl of 10Xbuffer, 3µl of 25mM MgCl₂, 1.25µl of 10mM dNTP’s, and 0.25µl of Taq polymerase and Finally 34.5µl of the free Dnase/Rnase water. Each PCR reaction was carried out using 5µl DNA and 45µl of the previously prepared Master Mix in a final volume of 50µl.

3.2.9.2 The PCR Conditions

The amplification conditions involved an initial denaturation step at 94°C for 10 min, 32 cycle denaturation at 94°C for 30 seconds, annealing at 57°C for 40 seconds, extension at 72°C for 50 seconds, that is followed by a final extension step at 72°C for 5 min and then cooled at 4°C for ∞. The PCR annealing temperature was considered according to the following equation: 4 * (C+G) + 2* (A+T) - 5°C.

The PCR products were detected in 1.5% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.

3.2.10 Detection of aac-6-Ib Gene

Multiplex PCR was utilized to detect the presence of aac-6-Ib gene. The DNA extraction was performed as described above.

3.2.10.1 The Master Mix and the Working Solution

The working solution of the PCR master mix was prepared by adding 0.3µl of each primer (the forward and reverse primers), 5µl of 10Xbuffer, 4µl of 25mM MgCl₂, 1.25µl of 10mM dNTP’s, and 0.25µl of Taq polymerase and Finally 38.9µl of the free Dnase/Rnase water. Each PCR reaction was carried out using 5µl DNA and 45µl of the previously prepared Master Mix in a final volume of 50µl.
3.2.10.2 The PCR Conditions

The amplification conditions involved an initial denaturation step at 94°C for 10 min, 35 cycle denaturation at 94°C for 1 min, annealing at 59°C for 1 min, extension at 72°C for 1 min, that is followed by a final extension step at 72°C for 10 min and then cooled at 4°C for ∞. The PCR annealing temperature was considered according to the following equation: 4 * (C+G) + 2* (A+T) - 5°C.

The PCR products were detected in 2% agarose gel in the presence of DNA ladder and loading dye, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.

3.2.11 Agarose Gel Electrophoresis

The agarose gel was prepared by suspending agarose at a concentration of 1.5% (w/v) in 150 ml of 1X TAE buffer. The agarose was heated in the microwave for 3 min until completely dissolved, then was cooled for 10-15 min, and poured into the mini electrophoresis cell. After 1 hour solidification of the gel, the comb and the gates were removed. A volume of 10µl of each DNA amplified product was mixed with 5µl of loading dye and applied to the gel. The PCR DNA ladder was also applied and the gel was run at 70 Volts for 1 hour, then placed in the Ethedium Bromide for at least 30 minutes and then photographed.
4 Statistical Analysis

SPSS Software (version 16.0) was used for data analysis including pearson correlation analysis. Pearson Correlation was used to indicate how the values of KPC Positive and aac-6'-ib Positive are related to each other in conjunction with an external factor (eg. Leipzig during the outbreak). The Pearson formula used is:

\[
r = \frac{\sum_{i=1}^{n}(x_i - \bar{x})(y_i - \bar{y})}{\sqrt{\sum_{i=1}^{n}(x_i - \bar{x})^2} \sqrt{\sum_{i=1}^{n}(y_i - \bar{y})^2}}
\]

Where \( r \) is Pearson correlation

\( Y \) is KPC Positive or aac-6'-ib Positive

\( X \) is the external factor (Leipzig Temperature Condition during the infection).
5 Results

5.1 Bacterial Isolates

During the period from January 2011 to October 2011, a total of 50 isolates of carbapenemases were collected from the University Hospital of Leipzig by the Microbiology department (Duplicate isolates were excluded).

5.2 Organisms Responsible for the Carbapenemase/QRDR-enzymes Production

Out of the 50 isolates that were found to be positive for carbapenemase and QRDR-producing organisms, 44 isolates were *Klebsiella pneumoniae* (88%), two *E. coli* (4%), two *Enterobacter cloacae* (4%), one *Proteus mirabilis* (2%), and one *Acinetobacter baumanii* (2%) (Fig: 10). The *E. coli*, *Enterobacter cloacae*, *Proteus mirabilis* and four of the *Klebsiella pneumoniae* isolates were KPC negative. The two aac-6´-Ib negative isolates were *Klebsiella pneumonia*.

![Carbapenemase/QRDR-producing organisms](image)

Figure 10: Carbapenemase/QRDR-producing organisms
5.3 The Source of the Clinical Isolates

Fifty clinical isolates of carbapenemase-producing organisms were collected from five major sources; Urine (16), Blood (5), swabs (9), wound (7), and other sources (13) which include: sputum (3), tracheal secretions (5), anal smears (3), and biopsies (2) (Fig: 11 and 12).

Figure 11: The source of the collected isolates

Figure 12: Other sources for the collected isolates
The major source of the study collected clinical isolates was from urine followed by swabs and wounds. Blood specimens had the lowest percentage among the major collected specimens.

Clinical isolates collected from other specimens; other than the major specimens (urine, swabs, and wound) were mainly collected from tracheal secretions followed by sputum and anal smears and finally biopsies.

5.4 Pulsed Field Gel Electrophoresis

All PCR products were examined using PFGE according to BIO-RAD CHEF genomic DNA plug Kit manual as described above. Results showed that 41 isolate showed the same patterns while 9 isolates showed different patterns (Fig: 13). After testing the presence of KPC gene by PCR test, the 9 isolates which showed different patterns were all KPC negative.

Figure 13: Pulsed Field Gel Electrophoresis for some of the isolates
5.5 Detection of KPC Gene by PCR

The KPC gene of 424 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 14).

![Image](image_url)

Figure 14: KPC (424 bp) gene electrophoresis. *(+): positive control, (-): negative control, L: Ladder

The 50 isolates on gel electrophoresis were not all positive for KPC, although the MIC values and the antimicrobial test showed multiresistance KPC. Nine isolates showed no bands on the gel and the test was repeated two times to confirm that these isolates are KPC negative. These negative KPC isolates showed different patterns on PFGE than the KPC positive. As a result, the prevalence of the KPC gene was 82%.

5.6 Detection of qnrA by PCR

The qnrA gene of 516 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 15 and 16).
Results showed that all the 50 isolates were negative for qnrA gene; no bands appear in the gel except the positive control which was strongly positive. The prevalence of qnrA gene among the study isolates were 0%.
5.7 Detection of qnrB Gene by PCR

The qnrB gene of 469 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 17).

Figure 17: QnrB (467 bp) gene electrophoresis: *(+): positive control, (-): negative control, L: Ladder

One of fifty isolate (isolate number 3) showed a band in the gel electrophoresis and was considered qnrB positive isolate. As a result, the prevalence of the qnrB gene was 2%. This isolate was collected from a urine sample and it was also a KPC positive isolate. Furthermore, this isolate also showed aac-6'-ib gene positive. So, isolate number 3 carried KPC, qnrB, and aac-6'-ib genes.

5.8 Detection of qnrS Gene by PCR

The qnrS gene of 417 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 18).
Results showed that all 50 study isolates were qnrS gene negative; no bands appeared in the gel electrophoresis. As a result, the prevalence of the qnrS gene was 0%.

### 5.9 Detection of IMP Gene

The IMP gene of 447 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 19).
Results showed that all 50 study isolates were IMP gene negative; no bands appeared in the gel electrophoresis. As a result, the prevalence of the IMP gene was 0%.

**5.10 Detection of VIM and OXA-48 Genes by PCR**

The VIM gene of 390 bp and the OXA-48 gene of 438 bp were amplified in all collected isolates by multiplex PCR as described above.
Figure 20: VIM (390 bp) and OXA-48 (438 bp) genes electrophoresis: *(+): positive control, (-): negative control, L: Ladder

The two genes were run at the same gel electrophoresis. Positive and negative controls were used. The band was detected for the gene and for the positive controls while none in the negative PCR blank (Fig: 20). Results showed that there was only one band appeared on the gel and it was the same size as the VIM positive control. As a result, isolate number one was VIM gene positive. It was collected from a blood culture specimen and it was also positive for aac-6’-ib gene but was KPC negative and it showed different patterns on PFGE. The prevalence of VIM gene was 2%. On the other hand, no bands appeared on the gel for OXA-48 gene. The prevalence of this gene was 0%.

5.11 Detection of NDM-1 Gene by PCR

The NDM-1 gene of 621 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 21).
Figure 21: NDM-1 (621 bp) and OXA-48 (438 bp) genes electrophoresis: *(+): positive control, (-): negative control, L: Ladder

Results showed that all 50 study isolates were NDM-1 gene negative; no bands appeared in the gel electrophoresis. As a result, the prevalence of the NDM-1 gene was 0%.

5.12 Detection of aac-6-Ib Gene by PCR

The aac-6-Ib gene of 482 bp was amplified in all collected isolates by multiplex PCR as described above. Positive and negative controls were used. The band was detected for the gene and for the positive control while none in the negative PCR blank (Fig: 22).

Figure 22: AAC-6’-Ib (482 bp) and OXA-48 (438 bp) genes electrophoresis: *(+): positive control, (-): negative control, L: Ladder

Results showed that 48 from 50 isolates were aac-6-Ib gene positive. Only two isolates were aac-6-Ib negative, isolate number 20 and 25. Isolate number 20 (urine specimen) was KPC, qnrA, qnrB, qnrS, IMP, VIM, NDM-1, OXA-48, and aac-6-Ib genes negative; this suggested that it has other multiresistance genes other than the study genes. Isolate number 21 (Swab
specimen) was negative for all the study genes except it showed a KPC positive gene. As a result, the prevalence of aac-6-Ib gene was the highest and recorded 96%.

### 5.13 Comparison the Prevalence of the Study Genes in the Different Isolate Sources

The KPC gene was detected in 82% of the isolates, while 8% were non KPC-producing organisms. Comparing the presence of KPC gene in the different isolate sources, swabs isolates were 89.0% KPC positive, followed by urine which was 87.5% of the isolates positive for KPC gene, then wounds isolates 76% and finally blood isolates 60% (Fig: 23).

![KPC Percentages](image)

Figure 23: The KPC positive isolates in the different specimens sources

The qnrA, qnrS, IMP, OXA-48, and NDM-1 genes were not found in any of the research isolates. Urine, blood, wounds, swabs, and the other research isolates were all negative for these genes.

The qnrB gene was detected in 2% of the isolates, while 98% were negative for this gene. Only one qnrB positive isolate was detected and was found in urine 6.25%. As for qnrB gene,
the prevalence for VIM gene was 2% of the isolates. The only positive VIM isolate was found in blood 20%.

The aac-6-Ib gene was detected in 96% of the isolates, while only 4% were negative for this gene. Comparing the presence of aac-6-Ib gene in the different isolate sources, blood, wounds, sputum, tracheal secretions, biopsies, and anal smears were 100% aac-6-Ib gene positive, followed by urine 94% and then swabs 89%.

In general, urine was found to have KPC, qnrB, and aac-6-Ib genes among its isolates, while blood was found to have KPC, VIM, and aac-6-Ib genes among its isolates. Wound, swabs, sputum, tracheal secretions, biopsies, and anal smears isolates were found to have only KPC and aac-6-Ib genes.

5.14 Relation between Patients Age and Carbapenemase / QRDR-Producing Organism’s Infections

Results showed that the median age of the carbapenemases and/or QRDR genes producing organisms infected patients, was 53 years. Only one case was a child who was born in 2010. Results in this outbreak suggested that the old aged patients were more likely to have the infection.

5.15 Relation between Patients Gender and Carbapenemase / QRDR-Producing Organism’s Infections

Male to female ratio was found to be 2,3:1. In KPC positive isolates the ratio was 3,1:1, while in aac-6’-Ib was 2,2:1. That means, in this outbreak male were more likely to have the infection than females (Fig: 24).
5.16 Relation between Weather Temperature and KPC / aac-6'-Ib

Genes Positive Isolates

Results showed that isolates (KPC and aac-6'-Ib genes positive) in this outbreak were mainly collected in winter time (low temperature). Most of them were from September to March. That means, in summer time (April to August) there were few cases of patients infected by KPC and aac-6'-Ib producing organisms (table 2).

![Figure 24: The infected male and female ratio](image)

<table>
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<th>Total Monthly aac-6'-ib Positive</th>
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Correlations

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**. Correlation is significant at the 0.005 level (2-tailed).

Table 2: Correlations for weather and infection relation
6 Discussion

Beta-lactamases are emerging worldwide as acquired resistance in Gram-negative bacteria such as in the Enterobacteriaceae (Bush, 2001). The Enterobacteriaceae multidrug resistance problem is obviously increasing and causing limited treatment options. The morbidity, mortality, and the associated economic burden are very likely to increase dramatically due to these organisms during the next decade (De Kracker et al., 2011).

6.1 The Study Isolates

In this study, we were looking for the presence of carbapenemase and QRDR genes (KPC, qnrA, qnrB, qnrS, IMP, VIM, NDM-1, OXA-48, and aac-6’-Ib) in clinical isolates collected during an outbreak in the University Hospital of Leipzig during the period from January 2011 to October 2011. Out of the 50 isolates, results showed that the main carbapenemase and QRDR-producing organisms (44 isolate) were *Klebsiella pneumoniae* (88%), followed by two *E. coli* isolates (4%), two *Enterobacter cloacae* (4%), one *Proteus mirabilis* (2%), and one *Acinetobacter baumanii* (2%).

Studies such as in Korea between 2004 and 2006, the qnrB gene was found mainly in *Citrobacter freundii* (67.9%) and *Klebsiella pneumoniae* (62.5%) followed by *Enterobacter cloacae* (15.8%) and *E. coli* (9.4%) (Tamang et al., 2008).

In Korea, another study between 2005-2006 detected the aac-6’-Ib gene mainly in *E. coli* (53.8%) followed by *Klebsiella pneumoniae* (19%) and then *Enterobacter spp* (12.9%) (Kim et al., 2009). Another study in Nigeria showed that the highest prevalence of carbapenemase was found in *Pseudomonas aeruginosa* (38.55%) followed by *E. coli* (34.8%), *Proteus mirabilis* (29.1%) and least prevalence in *Klebsiella pneumoniae* (25%) (table 3) (Yusuf et al., 2012).
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</table>

Table 3: Occurrence of Carbapenemase among the Clinical bacterial Isolates (Yusuf I et al., 2012).

### 6.2 The Source of the Study Clinical Isolates

In this study, the major source of the collected clinical isolates (50 isolates) was from urine (16), followed by swabs (9), wound (7), and then Blood (5). 13 isolates were collected from other sources such as sputum (3), tracheal secretions (5), anal smears (3), and biopsies (2). Multiple studies agreed with this result. In a study carried out by Manageiro et al. in 2012 to identify the resistance mechanisms of Enterobacteriaceae isolates, the majority of isolates were collected from the urine (57.4%) (Manageiro et al., 2012). Another study in Greece during a period of 3-years from January 2009 to December 2011, 73 carbapenemase-producing Klebsiella pneumoniae isolates were collected. The most common type of sample from which these pathogens were isolated was urine (50%), followed by bronchial secretions (17.8%), blood samples (14.3%), pus (13.1%), and intravenous catheters (4.8%) (Poulou et al., 2012).

On the other hand, a study carried out by Papaioannou et al. to determine the mechanisms of carbapenem resistance among multi-drug resistant Klebsiella pneumoniae (n=521) in a tertiary hospital, the study isolates were mainly collected from blood (119), followed by urine (97), then pus, catheters, and finally bronchial secretions (Papaioannou et al., 2013). Another study in South Africa carried out by Ehlers et al., the origins of the Acinetobacter baumannii isolates were 58% from sputum specimens, followed by 7% from urine specimens, 11% from blood cultures and 24% from diverse specimens (Ehlers et al., 2012).
In a study carried out in New York City, 19 isolates of *Klebsiella* spp producing carbapenem-hydrolyzing beta lactamase were recovered from November 1997 to July 2002 from 7 different hospitals. Eighteen were identified as *K. pneumonia*, and the remaining one was *K. oxytoca*. This study suggested the first cases of *Klebsiella pneumoniae* producing KPC to be detected in the United States (Bradford et al., 2004).

Another study at the same period time also in New York City was carried out during the period of April 2000 to April 2001. Twenty-four patients in intensive care units at Tisch Hospital were colonized by carbapenem-resistant *K. pneumonia*. Fourteen of the 24 patients were infected. Out of the fourteen infected patients, eight patients died. This *Klebsiella* infection was considered the cause of death. Furthermore, this study is considered the first cases of *Klebsiella* with this phenotype in the hospital (Woodford et al., 2004).

### 6.3 The KPC Gene

The KPC producers are found nowadays worldwide and considered endemic in several countries in Asia, South America, the Middle East, and Europe (Bush and Fisher, 2011, Nordmann et al., 2009).

In our study results concerning KPC gene showed that out of 50 samples, 41 isolate were positive for the KPC genes while 9 isolates were negative and showed different patterns on pulsed field gel electrophoresis. The prevalence of the KPC gene was 82%.

In China a surveillance study of carbapenem-resistant *Enterobacteriaceae* isolates was carried out in order to investigate the frequency and prevalence of KPC-type genes in Huashan Hospital. From January 2005 to March 2010, 109 nonduplicate *Klebsiella pneumoniae* isolates with resistance to ertapenem were collected. The presence of genes encoding β-lactamases, including KPC-type and OXA-type carbapenemases were screened using PCR method and all amplified products were then subjected to direct nucleotide sequencing. Results of this study reported for the first time the frequency of KPC-2-type carbapenemases
among carbapenem-resistant *K. Pneumoniae* isolates in China. Furthermore, results showed a significant increase in the incidence of carbapenem-resistant *K. pneumoniae* isolates from 0.9% in 2005 to 12.9% in 2009 which suggested being due to the failure to control the spread of these strains (Chen et al., 2011).

In a prospective study carried out in the period from August 2006 to November 2006, a total of 108 *Klebsiella pneumoniae* isolates were collected from a hospital in the United States in order to determine the prevalence and genetic relatedness of KPC positive strains. Results showed that the prevalence of KPC-possessing *Klebsiella pneumoniae* in this institution has risen to 9.2% in four months (Tibbetts and Dunne, 2007).

On the other hand, several outbreaks and sporadic cases of KPC-producing *K. pneumoniae* were reported in many places such as a study in Italy in 2009. Two cases isolates of carbapenem-resistant *K. pneumonia* obtained from patients at an Italian teaching hospital were collected. The first strain was obtained from a central venous device in a Crohn's disease patient. The second was isolated from a urine sample from a urinary catheter in an ICU patient with a haematoma. As a result, KPC-2 was identified. In addition, it was found that it was the second documented appearance of a class A carbapenemase-producing isolate of *K. pneumonia* in Italy and was not associated with travel abroad (Fontana et al., 2010).

Further cases were found in Greece in the period from February 2008 until December 2008. Twenty one hospitals provided a total of 225 *K. pneumonia* isolates which were identified as possible KPC-producing isolates. As a result, 77% isolates from 18 hospitals were found to harbour the KPC-2 gene. The remaining 52 isolates were found to harbour the VIM gene (Giakoupi et al., 2009).

### 6.4 The qnrA and qnrS Genes

Results of our study showed that all the study isolates were negative for the qnrA, qnrS, IMP, OXA-48, and NDM-1 genes. On the other hand, the qnr genes were detected worldwide, i.e.
in Korea (Tamang et al., 2008), Iran (Saboohi et al., 2012), Hungary (Szabo et al., 2008), and in many different places around the world.

A 368 clinical isolates were collected during 2004 to 2006 at Kyungpook National University Hospital in Korea. As a result, 141 (38.3%) isolates were qnr positive. The qnrA, qnrB, and qnrS genes were detected in 1.0%, 36.7%, and 0.5% of the isolates, respectively. These results agreed with our results in qnrB high percentage while qnrA and qnrS are very close to our results which showed 0% of these genes (Tamang et al., 2008).

In Hungary 2002 until 2006, clinical isolates of 70 E. coli, 101 K. pneumonia, 5 Citrobacter freundii and 61 Enterobacter spp isolates were collected from two laboratories in Budapest to study the prevalence of qnrA, qnrB, qnrS and aac(6’)-Ib-cr genes. As a result, the prevalence was low: around 3% for qnrA, 0.8% for qnrB, 0.4% for qnrS and 8% for aac(6’)-Ib-cr genes (Szabo et al., 2008).

In a study in Germany carried out during the period of 2000 to 2003 in Enterobacter spp and Citrobacter freundii isolates the qnrA gene was detected but the prevalence was still low; among 703 clinical Enterobacteriaceae isolates from 34 German ICUs which showed resistance to cephalosporin or fluoroquinolone, four patients were qnrA gene positive (Jonas et al., 2005).

Another research in the United Kingdom in Liverpool studied the prevalence of qnrA gene in 47 blood culture isolates of Enterobacteriaceae. These strains were resistant to ciprofloxacin and cefotaxime. Results showed that the prevalence of the qnrA gene among these isolates were 32% and they included isolates of E. coli, Citrobacter freundii, Klebsiella pneumoniae and Enterobacter cloacae. This report was the first to address the qnrA gene identification in the United Kingdom and the prevalence was considered to be high (Corkill et al., 2005).

In a study carried out by Saboohi and his colleagues, a total of 85 of isolates of Salmonella spp. were collected from different provinces of Iran during the years 2008 to 2010. Results showed the presence of qnrA, qnrB, and qnrS genes among the isolates. 22 of 85 (25.8%)
carried the qnrA gene, 1 (1.17%) qnrB and 1 (1.17%) qnrS gene and 1 isolate carried all the three genes. Furthermore, the resistance to quinolones and fluoroquinolones by these genes has been confirmed (Saboohi et al., 2012).

In August 2012, a study described the prevalence of qnr determinants (qnrA, qnrB, and qnrS) in 19 fluoroquinolone-resistant *Escherichia coli* isolates from chicken litter. Results suggested the presence of qnrS and qnrA genes in these fluoroquinolone-resistant isolates. It has been suggested that the emergence and dissemination of fluoroquinolone resistant strains can be from the contaminated poultry by-products and be acquired by human beings via the food supply (Ponce-Rivas et al., 2012).

In a collection of Enterobacterial isolates collected from the Bicêtre hospital In France, a study was carried out to evaluate the prevalence of qnrA and qnrS genes. In this study the prevalence of the qnrA determinant was low (Poirel (B) et al., 2006).

### 6.5 The NDM-1 Gene

Comparing our results that showed no NDM-1 gene and low prevalence of VIM gene, a study was carried out in the period between April 2009 and February 2011 in Mubarak Al Kabeer Hospital in Kuwait disagreed with our results. This study aimed to identify the mechanisms of carbapenem resistance among fourteen clinical isolates. Eleven out of fourteen isolates produced VIM-4 in *Klebsiella pneumoniae, E. coli, Enterobacter cloacae,* and *Klebsiella oxytoca* while three isolates were NDM-1 positive in *Klebsiella pneumoniae* (Jamal et al., 2013).

Multiple reports showed infected cases with NDM-1 positive organisms from patients who were in Indian subcontinent before, such as in the United Kingdom (Muir and Weinbren, 2010). Enterobacteriacea isolates were studied from south India, north India, and from the UK’s national reference laboratory. 44 isolates with NDM-1 were identified in south India.
(Chennai), 26 in north India (Haryana), 37 in the UK, and 73 in other sites in India and Pakistan (Kumarasamy et al., 2010).

In Australia, a multidrug-resistant *E. coli* isolate was recovered from a urine sample of a 67-year-old man who had been hospitalized at St. George Hospital in Sydney. This patient was previously hospitalized in Bangladesh and then transferred to Australia. Molecular investigations revealed the first identification of the NDM-1 gene in Australia (Poirel (B) et al., 2010).

In India 2010, three carbapenem resistant *Acinetobacter baumannii* were isolated from patients in the ICU in a hospital in Chennai (Karthikeyan et al., 2010). As stated in this study, it the first report of the existence of NDM-1 gene in clinical isolates of *Acinetobacter baumannii* in India. Further reports were from the United States (Centers for Disease Control and Prevention, 2010).

Investigations revealed that the NDM-1 gene was found in numerous clinical isolates from both nosocomial- and community-acquired *Klebsiella pneumoniae* and *E. coli* infections that were obtained from a wide geographic area of India subcontinent (Kumarasamy et al., 2010). Agreeing with our results in the absence of NDM-1 gene, a study used the PCR method to investigate the presence of the NDM-1 gene in a 210 clinical isolates in tertiary hospitals in Samsun, Turkey. The isolates included different organisms such as *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Pseudomonas putida*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Klebsiella pneumoniae*, *Providencia rettgeri*, *E. coli*, and *Citrobacter freundii*. As a result, the NDM-1 gene was not detected in any of the clinical isolates. This study together with other studies indicated the uncommon presence of the NDM-1 gene in Turkey (Yanik et al., 2013).
6.6 The OXA-48 Gene

Our study showed no OXA-48 gene among the study isolates. On the other hand, Reports showed that OXA-48 producing organisms were detected such as in Tunisia (Ktari et al., 2011), France (Cuzon et al., 2011), Morocco (Benouda et al., 2010), and Senegal (Moquet et al., 2011).

In Tunisia between the period of October 2009 until March 2010, 153 clinical isolates were recovered from Sfax University Hospital. These isolates showed reduced susceptibility to extended-spectrum cephalosporins and/or imipenem. Results showed 21 isolates from 153 (13.7%) were harbouring the OXA-48 gene (Ktari et al., 2011).

In France from April 2010 to June 2010, 260 patients hospitalized in the ICU and the internal medicine unit at the hospital of Villeneuve-Saint-Georges were screened. Seven patients were infected by multidrug-resistant K. pneumonia isolates and harboired the OXA-48 gene and 3 were only colonized. Researcher of this study suggested a nosocomial transmission since in April 2010 only three patients were detected to be infected with a multidrug-resistant K. pneumonia. The remaining cases were in contact with these cases and the infection was transmitted (Cuzon et al., 2011).

The first detection of the OXA-48 gene in Enterobacteriaceae in Morocco was reported in a study carried out in 2010, where one patient was infected with carbapenem-resistant K. pneumonia. From this patient, two isolates were recovered and screened. Results showed that one of the isolates carried the OXA-48 gene while the other harboured another gene that was the OXA-1 gene (Benouda et al., 2010).

Outbreaks of OXA-48-producing K. pneumoniae and other enterobacterial isolates have been also described. In the United Kingdom between January 2008 and April 2010, 20 K. pneumonia carbapenem resistant isolates were collected from 13 patients. The prevalence rate was found to reach 0.17%. The researcher reported this as an early and prolonged outbreak in the UK of K. pneumonia producing OXA-48 gene (Thomas et al., 2013).
In a single case study in Lebanon 2008, a 7-year-old female child complained from a prolonged urinary tract infection. *K. pneumonia* isolate showed multidrug resistance. PCR results were negative for TEM, SHV, CTX-M, GES, KPC, IMP, VIM, IMI, SPM, GIM, and SIM genes. The OXA-48 gene was the only positive gene that was found in this isolate. Researcher of this study considered this study as the first evidence of beta lactamase mediated carbapenem resistance in Enterobacteriaceae in Lebanon (Matar et al., 2008). The OXA-48 gene was also detected in India in a study was described in a conference 2010 (Bell et al., 2010).

The OXA-48 producing organism was first identified in *Klebsiella pneumonia* clinical isolate in Turkey in 2004. The *K. pneumonia* was isolated in September 2001 at the Istanbul Faculty Hospital from a 54-year-old man with urinary tract infection and skin burns. This isolate was found to be resistant to all beta lactams, including carbapenems (Poirel et al., 2004).

Also in Istanbul, Turkey 38 isolates were collected at the University Hospital of Istanbul in the period from May 2006 to February 2007. Results showed that all isolates were positive for the OXA-48 gene. This study indicated the first large outbreak of OXA-48 positive carbapenem-resistant *Klebsiella pneumoniae* isolates (Carrer et al., 2008).

Another study was carried in Germany in 2012 for nine carbapenem resistant isolates collected from patients in five German hospitals. Results showed that six isolates were positive for the OXA-48 gene. Using the PFGE technique, a clonal transmission of an OXA-48 producing *Klebsiella pneumoniae* strain was indicated in two hospitals. Considering the high frequency of population exchanges between Germany and Turkey, in addition to the fact that Turkey represents a main reservoir for the OXA-48 carbapenemase, this study speculated that at least some of the isolates emerging in Germany could be originated from Turkey (Pfeifer et al., 2012).
6.7 The IMP Gene

Our study showed no occurrence of IMP gene among the isolates. A study carried out in 2000 by Takahashi A et al. at Gunma University Hospital disagreed with this result. Nine isolates from different inpatients were examined for the IMP gene by PCR method. All nine isolates were positive for this gene (Takahashi et al., 2000).

Another study tested a total of 58 clinical isolates for the presence of KPC, GES, IMP, VIM, OXA-48 and NDM-1 genes. Thirty were detected to be positive for these genes by PCR method. These positive strains included 21 *Enterobacteriaceae*, 1 *Acinetobacter baumannii* and 8 *Pseudomonas aeruginosa* isolates (Monteiro et al., 2012).

On the other hand, Moquet O et al., 2011 had the same result as in our study. November 2008 to October 2009, eleven *Enterobacteriaceae* isolates were collected at the Institut Pasteur in Senegal for the presence of VIM, IMP, KPC, OXA-48 genes as well as CTX-M, OXA-1, and TEM beta lactamase genes, the aac(6′)-Ib aminoglycoside resistance gene and the quinolone resistance genes qnrA, qnrB, qnrS. These isolates included 8 Klebsiella pneumoniae, 1 E. coli, 1 *Enterobacter cloacae*, and 1 *Enterobacter sakazakii*. The OXA-48 was detected in all 11 isolates. The qnr and aac(6′)-Ib genes were detected in 7 isolates, while VIM, IMP and KPC genes were not detected (Moquet et al., 2011).

6.8 The qnrB Gene

The qnrB gene in our isolates was found in one isolate out of fifty collected. So the prevalence of this gene was 2% and is considered low comparing to other studies. This isolate was found to carry in addition to the qnrB gene, the KPC gene as well as the aac-6′-Ib gene.

In a study carried out in 2009 in Peru and Bolivia, a relatively high prevalence of qnrB (54%) and low qnrS (14%) was detected in commensal Enterobacteria from 310 healthy children. As they mentioned in this study, it was the first study on the prevalence of qnr genes in human
commensal bacteria and suggested that these bacteria could be an important reservoir of similar genes (Pallecchi et al., 2009).

In another study carried out in Argentina in June 2013, the prevalence of plasmid mediated quinolone resistance (PMQR) genes such as qnrA, qnrB, qnrS, aac-6'-Ib-cr and other genes was investigated among 55 oxyiminocephalosporin-resistant enterobacteria collected in a previous survey. The main PMQR determinants were aac-6'-Ib-cr and qnrB in prevalence rates of 42.4% and 33.3% respectively (Cruz et al., 2013).

On the other hand, qnrB gene was detected in a low prevalence in a study carried out between 2005 and 2006. A total of 179 Gram negative bacterial strains collected in China were screened for qnrA, qnrB, and qnrS genes by using the PCR method. The qnrB gene was detected in 6.2% of *E. coli* and 7.69% of *E. cloacae* (Cai et al., 2011).

Another study was carried out in Budapest, Hungary, to determine the prevalence of qnrA, qnrB, qnrS and aac-6'-Ib-cr genes in ESBL-producing isolates. A total of 70 isolates were *E. coli*, 101 were *Klebsiella pneumoniae*, 5 were *Citrobacter freundii* and 61 were *Enterobacter spp*. These isolates were collected from seven different hospitals and clinics between the period from 2002 to 2006. As a result, the qnrB gene was detected only in two isolates: one *Klebsiella pneumoniae* and one *Citrobacter freundii* (Szabo et al., 2008).

### 6.9 The VIM gene

As a result of our study there was only one isolate which carried the VIM gene. This isolate was collected from a blood culture specimen and it was also positive for aac-6'-Ib gene but was negative for the KPC gene. By doing the PFGE, it showed different patterns than the KPC positive isolates. The prevalence of VIM gene was found to be 2%.

In 1999, VIM-1 as a new type of acquired metallo beta-lactamase was reported to have been found in an isolate of *P. aeruginosa* in Italy (Lauretti et al., 1999). VIM-2 isolates were detected in France in 1996 (Poirel et al., 2000; Poirel et al., 2001).
In October 2003, the first isolation of a VIM-2 from a Pseudomonas aeruginosa isolate in Germany was in a study carried out in Berlin from a blood culture of a 70-year-old male cancer patient during an episode of febrile neutropenia that followed a course of anticancer therapy. Positive result was identified for the presence of VIM gene. As the study researchers mentioned, this was the first isolation of the VIM gene in Germany (Henrichfreise et al., 2005).

The VIM gene was also detected in Greece in a study between 1996 and 1998. A total of 1,276 clinical isolates of P. aeruginosa were collected from 973 patients at a Greek university hospital. Among those, 211 isolates were resistant to imipenem and meropenem. In sex isolates the resistance was associated with the production of VIM-1 gene (Tsakris et al., 2000).

An outbreak was also reported in Greece, at the University Hospital of Thessaly, between the period of 2001 to 2002 involving 47 VIM-producing Pseudomonas aeruginosa isolates showed that the VIM-4 gene had been identified (Pournaras et al., 2002; Pournaras et al., 2003). While VIM-3 was reported in Taiwan in 10 clinical pseudomonas spp isolates out of 209 collected isolates (Yan et al., 2001). The VIM-4 was detected in the United States as Tolman and his colleagues (Tolman et al., 2002). The VIM-11 was detected in a study in Argentina November 2002. The study isolate was obtained from the catheter of a 7-month-old patient at the Hospital de Niños (Pasteran et al., 2005).

In a study in 2008, the prevalence of ESBL and MBL antibiotic resistance genes in Klebsiella pneumoniae isolates were investigated in isolates collected from Pretoria Academic Hospital in South Africa. A PCR assay was used for the detection of four genes which include CTX-M, SHV, TEM and VIM genes in 97 Klebsiella pneumoniae isolates. Results showed that the VIM gene was not detected in any of the isolates analysed in this study (Maningi et al., 2008).

On the other hand, a study was carried out in the largest teaching hospital (Mubarak Al Kabeer hospital) in Kuwait for fourteen carbapenem-resistant Enterobacteriaceae isolates
between April 2009 and February 2011. Results showed high VIM gene prevalence; Eleven out of fourteen produced the VIM-4 gene. It was found that six were in *Klebsiella pneumoniae*, three in *E. coli*, One in *Enterobacter cloacae* and one in *Klebsiella oxytoca*. Furthermore, the prevalence of NDM-1 gene was found to be low. Only three *Klebsiella pneumoniae* isolates produced the NDM-1 gene (Jamal et al., 2013).

Another study was carried out in Saudi Arabia for a total of 350 clinical isolates of *P. aeruginosa* to determine the production of metallo beta-lactamase (MBLs) using PCR and DNA sequencing assays. As a result, all the study isolates harbour the VIM-2 gene that suggested the VIM gene as the dominant MBL gene in MBL-producing isolates in Saudi Arabia (Al-Agamy et al., 2011).

Shehabi and his colleagues in 2011 isolated strains from effluent water were highly polluted with VIM producing *E. coli* and *P. aeruginosa* similar to those observed frequently in clinical bacterial isolates. They suggested that his might contribute to increase accumulation of antimicrobial resistance in the natural environment (Shehabi et al., 2011).

**6.10 The aac-6-Ib Gene**

Our study results showed that 48 from 50 isolates were aac-6-Ib gene positive. Two isolates only were aac-6-Ib negative; one was isolated from urine and was negative for all study genes. The other one was isolates from a swab specimen and was positive only for the KPC gene. As a result, the prevalence of aac-6-Ib gene was the highest and recorded 96%.

In a study carried out in Korea by Kim YT and his colleagues, reported the first published investigation in Korea of strains that included both aac(6')-Ib and aac(6')-Ib-cr variant. 85 strains of suspected aac(6')-Ib positive isolates were collected. Among them, 38 strains were harbouring the aac(6')-Ib; the remaining 47 strains were harbouring the aac(6')-Ib-cr variant. Of these 47 strains, 19 harbour aac(6')-Ib and aac(6')-Ib-cr simultaneously (Kim et al., 2011).
The aac(6′)-Ib gene was found to be detected worldwide, as discussed below, such as in the United States (Park et al., 2006), Italy (Frasson et al., 2011), Israel (Chmelnitsky et al., 2009), Brazil (Paiva et al., 2012) and many other places.

In a study in the United States during the period from January 2005 to December 2006, 555 Enterobacteriaceae isolates were examined for harbouring the aac(6′)-Ib gene. A total of 86 isolates carried the aac(6′)-Ib gene, while 19 were positive for the aac(6′)-Ib-cr gene. Further investigation showed that one aac(6′)-Ib positive K. pneumonia isolate carried both aac(6′)-Ib-cr and qnrS genes (Park et al., 2006).

Frasson and his colleagues studied a total of 197 enterobacterial isolates between the period of March 2008 until May 2008 for the presence of the aac(6′)-Ib-cr gene recovered in an Italian teaching hospital of Padua, Italy. Twenty five isolates (13%) were found to harbour the aac(6′)-Ib gene and of these 16 (8%) harboured the aac(6′)-Ib-cr gene variant. It was found that the aac(6′)-Ib-cr gene was exclusively found in E. coli strains, while aac(6′)-Ib gene was found mainly in K. pneumonia in 8 isolates and E. coli in 1 isolate (Frasson et al., 2011).

In Israel between 2004 and 2006, 47 KPC-producing K. pneumonia isolates were collected in Tel Aviv Medical Center. PCR experiment detected the aac(6′)-Ib-cr gene variant in six of the isolates which contributed 12.8% (Chmelnitsky et al., 2009).

Another study was in Brasil between May 2009 to November 2009. 101 ciprofloxacin-resistant E. coli isolates were collected in Belo Horizonte, Brazil. As a result, eight isolates harboured the qnr and the aac(6′)-Ib-cr genes. The study researchers reported these results as the first qnrB19, qnrS1 and aac(6′)-Ib-cr carrying E. coli isolates in Brazil (Paiva et al., 2012).

In a study carried out in Bulgaria in a period between 2000 and 2005 to investigate the prevalence of aac-6′-Ib-cr gene among a total of 163 ESBL-producing enterobacteria. Of these, 52 isolates (52.5%) were aac-6′-Ib-cr positive and it was considered the first report of qnrB and aac-6′-Ib-cr in clinical Enterobacteriaceae isolates from a Bulgarian hospital (Sabtcheva et al., 2009).
Another study was carried out in China to investigate the prevalence of qnr and aac-6\(^\text{Ib}-\text{cr}\) genes. A total of 265 clinical isolates were screened at nine teaching hospitals in China. These isolates include *E. coli*, *Klebsiella pneumoniae*, *Citrobacter freundii*, and *Enterobacter cloacae*. The qnrA, qnrB, qnrS, and aac-6\(^{\text{Ib}}\)-cr genes were detected by PCR assay. Results indicated the high prevalence of qnr and aac-6\(^{\text{Ib}}\)-cr genes among the *Enterobacteriaceae* (Yang et al., 2008).

On the other hand, among 555 nonduplicate enterbacterial blood culture isolates collected at Asan Medical Center in Korea, the aac-6\(^{\text{Ib}}\) was detected in 15.5% *Enterobacteriaceae* isolates. This is considered low prevalence comparing to our results for the same gene (Kim et al., 2009).

### 6.11 The Median Age

This research studied the median age of the carbapenemases and/or QRDR genes producing organisms infected patients. Results showed that the median age was 53 years (born in 1958). Only one case was for a child who was born in 2010. Figure 25 showed the percentages of isolates that is related to a patient age. According to these results we suggested that the old aged patients were more likely to have the infection.
Figure 25: The percentages of isolates related to a patient’s age

In a study included six out of seven patients with bloodstream infections caused by KPC-producing Klebsiella pneumoniae was carried out during the period from January to April 2005 in the ICU of a hospital in Manhattan. The patient’s median age was found to be 68 years (Nadkami et al., 2009).

In Chikago, swab samples from 33 patients were cultured for the detection of KPC gene. Results showed that the mean age of the patients infected with KPC-producing organisms was 66 years (Thurlow et al., 2013).

Another study carried out in New York City, nineteen isolates of KPC-producing Klebsiella spp were recovered from 7 hospitals. The median age of the 17 patients whom possessed KPC gene was 73 years (Bradford et al., 2004).

Very close to our result, a study carried out in Brazil for a 43 isolates of carbapenem resistance Klebsiella pneumoniae- producing organisms, the median age of the patients who presented with KPC infection was 54.5 years (range, 37–74 years) (Bergamasco et al., 2011).

As a conclusion in most studies, infections with carbapenemases as well as QRDR producing organisms were rare in children and adults and were most commonly found in aged patients as they considered having lower immunity. Figure 26 summerizes the common patient ages who were infected with Multidrug resistant organisms in some studies in different countries around the world.
6.12 Male to Female Ratio

Further results in our study indicated male to female ratio to be 2.3:1. In KPC positive isolates the ratio was 3.1:1, while in aac-6’-Ib was 2.2:1. That means, in this outbreak male were more likely to have the infection than females.

In Greece, a study carried out in the period from October 2007 to September 2008 for 47 patients showed that patients had a male to female ratio of 1.5 and a mean age of 59.5 years (Pournaras et al., 2009).

On the other hand, 77 cases reported in questionnaires in Europe, 51 were originated from the UK. The male to female ratio was 0.62 and the patients’ age ranged from 2 to 87 years (Struelens et al., 2010).

In Northeast India a study indicated that out of a 219 isolates, 19 were detected to produce carbapenemase. The male to female ratio was found to be 1.37:1 and the patients’ age ranged from 5 days to 75 years (Bora and Ahmed, 2012).
6.13 The Study of Seasonal Variation

In our study, results showed that isolates in this outbreak were mainly found in winter season (from September to March). While in summer season (April to August) there were only few cases (Fig 27). Infections caused by carbapenemases and QRDR-producing organisms were detected in winter time in some studies and in summer time in other studies.

Figure 27: The relationship between Weather Temperature and KPC and aac-6'-ib positive genes

A study was carried out from blood stream infections caused by KPC-producing Klebsiella pneumoniae in ICU in Manhattan. These isolates were collected during the winter time from January 2005 to April 2005 (Nadkami et al., 2009). Another study was carried out in the USA at the end of summer and the beginning of winter time, from August 2006 to November 2006 (Tibbetts and Dunne, 2007).

On the other hand, studies isolates were collected in summer or relatively high temperature time. Kim SY and his colleagues detected the aac-6'-Ib gene in isolates collected in a period between March to July 2005 (Kimm et al., 2010).
Scientists from The Oregon State University, the University of Maryland School of Medicine, and researchers from the University of Florida and the Research Institute of the Hospital for Sick Children in Toronto found that bacterial infections increase during the summer time. They suggested but not proved that these findings were due to many reasons; for example *P. aeruginosa* infections could be linked to more people swimming in lakes or pools during the summer. Furthermore, Cattle have higher bacterial shedding rates in the summer, and the peak of *E. coli* infections could be connected to higher consumption of ground beef or other factors during the "outdoor grill" season (Oregon State University, 2008).

### 6.14 Additional Study Isolates Relationships

Further statistical analyses were used to show the number of isolates which (table 4):

1. carried the KPC gene and were negative for the aac-6'-ib gene.
2. carried the aac-6'-ib gene and were negative for the KPC gene.
3. Carried both the KPC and aac-6'-ib genes simultaneously.

<table>
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Table 4: The KPC and aac-6'-ib genes Crosstabulation

### 6.15 Prevalence of Carbapenemases and QRDR Genes

Data from reported to the CDC from 2007 indicated that 8% of all Klebsiella isolates were carbapenem-resistant K. pneumoniae (CRKP), in comparison with <1% in 2000 (Srinivasan
and Patel, 2008). Since 2008, four different bacterial strains (3 Enterobacter cloacae and 1 Klebsiella pneumoniae) were isolated from the rectal swabs of 2 patients admitted to the Caritas Baby Hospital (CBH) in Bethlehem, Palestine. These isolates were resistant to at least one of the carbapenems (ertapenem, imipenem, and meropenem). All isolates were KPC-2 positive and showed the presence of different combination of plasmid encoded beta-lactamase genes such as TEM, SHV, OXA-1, MIR-1, GES-23, and KPC-2 in these isolates. These isolates were resistant to all beta-lactam antibiotics, co-trimoxazole, and gentamycin (Kattan et al., 2012).

During the period of 1995-2002, isolates were collected at the tertiary-care hospital in Korea to investigate the phenotypic and genetic properties of metallo beta-lactamase producing Pseudomonas isolates. The prevalence of imipenem resistance (IMP) among Pseudomonas aeruginosa isolates reached 16% in 1997, when 9% of the resistant organisms were found to produce VIM-2 β-lactamase (Lee et al., 2002).

A multicenter study in Greece reported that 62.1% of imipenem-resistance P. aeruginosa strains were VIM-2-producers and were detected in nine of the 15 hospitals examined in 2001 (Giakkoupi (B) et al., 2003).

In a study carried out in Iran in 2013, 100 P. aeruginosa isolates were included in this study. 83 isolate (83%) were resistant to imipenem. Among these resistant isolates 48 (57.9%) were metallo beta-lactamase producers. Using PCR 6 isolates were positive for IMP gene type IMP-1, while no VIM gene was detected (Fallah et al., 2013).

In 2012, a study was carried out in Northeast India for 219 K. pneumoniae isolates showed that a total of 19 isolates were screened for carbapenemase production. All the screening positive isolates (n =19) were found to be positive for the plasmid mediated blaNDM-1 gene by PCR. Out of these 19 isolates 57.89% were from urine, 15.79% were from sputum, 10.53% were from blood and 15.79% were from pus. The screening positive isolates were also found to be positive for the modified Hodge test. The age of the patients with the NDM-1 positive
isolates ranged from 5 days to 75 years and the male to female ratio was 1.37:1 (Bora and Ahmed, 2012). Due to lack of epidemiological data within India, the exact prevalence of NDM-1 enzyme is not known.

From October 2009 to March 2010, 153 clinical isolates of *K. pneumoniae* with reduced susceptibility to extended-spectrum cephalosporins and/or imipenem were collected from Sfax University Hospital, Tunisia. Among these isolates, 21 (13.7%) was found to be blaOXA-48 gene producers. In 20 OXA-48-producing isolates, the blaOXA-48 gene was observed to be transferable to *Escherichia coli* strain. The only one remaining OXA-48 positive isolate was suggested to be chromosomally located (Ktari et al., 2011).

In a study carried out at Kyungpook National University Hospital in the Republic of Korea, 368 non duplicate clinical isolates of *Enterobacteriaceae* were collected during the period of 2004 to 2006 which were resistant to nalidixic acid and at least to one of the extended-spectrum-beta-lactams (cefoxitin, cefotaxime, cefepime, aztreonam, or ceftazidime). Results showed that a total of 141 (38.3%) isolates were qnr positive. The qnrA (1.0%), qnrB (36.7%), and qnrS (0.5%) were detected but none of the isolates had two or more types of these genes simultaneously. Furthermore, results detected the qnrB mostly in *Citrobacter freundii* isolates (67.9%), followed by *K. pneumoniae* (62.5%), *Enterobacter cloacae* (15.8%), and *Escherichia coli* (9.4%) isolates. While qnrA was detected only in *E. cloacae* isolates and qnrS was detected in one *K. pneumoniae* and one *E. coli* isolate (Tamang et al., 2008).

Another study for identification of Qnr genes to detect qnrA, qnrB, and qnrS genes was carried out in a Taiwanese university hospital in 2004. 526 nonreplicate clinical isolates of *Enterobacter cloacae* were collected. Results showed that eighty-six (16.3%) of all isolates were qnr positive; qnrA1 (0.6%), qnrB1 (10.1%), and qnrS1 (6.5%) (Wu et al., 2007).

During the period from January 2005 to December 2006, a study was carried out at Asan Medical Center in Korea to detect the presence of aac-6-Ib gene among 555 nonduplicate
enterobacterial isolates collected from blood cultures. The aac-6′-Ib gene was detected in 15.5% *Enterobacteriaceae* isolates. Among these aac-6′-Ib-positive isolates, 12.9% were *Enterobacter spp.*, 53.8% *Escherichia coli* isolates, 19.0% *Klebsiella pneumoniae* isolates. They suggested that there was a strong association between aac-6′-Ib-cr and OXA-1 and CTX-M-1 group β-lactamase genes. Furthermore, they investigated that one aac-6′-Ib-positive *K. pneumoniae* isolate carried both aac-6′-Ib-cr and qnrS (Kim et al., 2009).

### 6.16 Epidemiology of Carbapenemases and QRDR Genes

#### 6.16.1 Europe

In 2005, the first report of a clinical isolate producing a KPC outside of the USA occurred in France from a patient who had been hospitalized in New York City (Naas et al., 2005). KPC-producing isolates have been reported from several European countries including Poland. A single case study was reported on a 56-year-old patient with no travel history who was admitted to a cardiology ward in a Warsaw hospital. PCR and sequencing identified the presence of KPC-2 gene (Baraniak et al., 2009). In the UK, 2 isolates were studied. The first *K. pneumoniae* was isolated in 2007 from an elderly male in a Scottish hospital. The second isolate was from London in 2008 from an elderly female who was admitted into a hospital in Israel. The KPC gene was detected by PCR in both isolates (Woodford et al., 2008).

In areas such as Greece where KPCs are now considered endemic, many outbreaks have occurred (Nordmann et al., 2009). Epidemic situations of KPC-2 have also been reported in Greece in a 12-month period study. Between the period of October 2007 to September 2008, 47 clinical isolates were obtained from Hippokration University Hospital for a *K. pneumoniae* strains that showed reduced susceptibility to carbapenems and were positive for carbapenemase production. PCR results showed that all isolates carried the KPC-2 gene (Pournaras et al., 2009).
Another study in Greece was a single case study in 2007 for a 22 year-old woman who was hospitalized in the ICU of the hospital of Heraklion for severe cranial trauma with coma. PCR and sequencing identified beta lactamase genes coded for KPC-2 and TEM-1 (Cuzon et al., 2008).

The Enterobacteriaceae-producing KPCs have also been recently reported in Italy and Finland in a research that studied two cases. The first case was a patient transferred from Greece to Finland in June 2009. This isolate was a K. pneumoniae strain. PCR and sequencing confirmed harbouring of the KPC-2 gene. Case two was for a patient who was transferred from north-western Italy to Finland in August 2009. By PCR and sequencing, the isolate was found to harbour the KPC-2 gene (Osterblad et al., 2009).

In Germany in the period from July 2010 to January 2011, an outbreak of KPC-2 and VIM-1 was reported in the ICU department of the University Hospital Essen. Carbapenem-resistant K. pneumoniae strains were isolated from seven patients (5 men and 2 women). The patients age ranged from 22 to 72 years. Three of four patients were died from these strains. As the study researchers mentioned, this report was the first of the detection of a carbapenem-resistant Enterobacteriaceae producing KPC-2 and VIM-1 outside of Greece (Steinmann et al., 2011).

The VIM was first isolated in Verona, Italy, in 1997. The study was a single case study for a clinical isolate from an Italian inpatient at the Verona University Hospital. This isolate was a carbapenem-resistant P. aeruginosa. Sequencing of the gene showed that it harbour a new class B beta lactamase that was then named VIM-1 (Lauretti et al., 1999).

The fluoroquinolone resistance was reported in many studies; in Barcelona, Spain during the period from 1992 to 1997, a study carried out on the evolution of quinolone resistance revealed that the prevalence of fluoroquinolone resistance in the feces of healthy people was high, 24% in adults and 16% in children (Garau et al., 1999; Oteo et al., 2005).
The aac(6′)-Ib gene was found to be detected worldwide such as in Italy in a study discussed previously (Frasson et al., 2011).

### 6.16.2 The Americas

To date, KPC-producing bacteria have been isolated in at least 33 states (Kitchel et al., 2009). The first KPC isolates *Klebsiella pneumoniae* was found in the United States in North Carolina in 2001 and then spread in New York (Yigit et al., 2001; Patel et al., 2009; Kitchel et al., 2009).

KPCs and its derivatives were also considered endemic according to many outbreaks in many areas in USA such as the north-eastern USA (Nordmann et al., 2009). A citywide surveillance study was carried out in Brooklyn. A total of 602 isolates were collected from an outbreak in 2 hospitals. Of 602 isolates of *K. pneumoniae* collected, 45% had ESBLs. Of the ESBL producing isolates, 3.3% harboured the KPC-2 gene (Bratu (B) et al., 2005).

In New York, 3 patients cases were studied: the first patient was a 44-year-old man recovering from a kidney transplantation in January 2003. PCR confirmed that this isolate harboured the KPC-2, TEM-1, and SHV-12 genes. The second patient was a 39-year-old man who experienced multiple trauma in April 2005. PCR showed that this isolate carried the KPC-2 gene. Finally, the third patient was a 41-year-old woman who was at the end-stage of renal disease. After her hospital admission, *K. pneumoniae* was identified. PCR analysis confirmed that this isolate harboured the KPC-2 gene (Lomaestro et al., 2006).

In the ICUs of a hospital in Manhattan, seven patients with bloodstream infections caused by *K. pneumonia* isolates were identified between January 2005 and April 2005. PCR confirmed that all isolates harboured the KPC gene and sequencing identified the KPC-2 gene. Further results showed that the median age was 68 years (Nadkarni et al., 2009).

In a study carried out by Park and his colleagues, the *aac(6′)-Ib* gene was found in 50.5% of isolates, and of these, 28% carried the *cr* variant (Park et al., 2006).
6.16.3 Africa and Middle East

The NDM-1 (New Delhi metallo-beta-lactamase) gene containing organisms have emerged and are now spreading in all continents. The first reported case of the NDM-1 (name New Delhi) was in the Indian origin of which was in 2009 in a Swedish patient who travelled to New Delhi and acquired a UTI caused by carbapenem resistant *Klebsiella pneumoniae* and E. coli strains (Yong et al., 2009).

In a study carried out by El-Herte and his colleagues, three patients from Iraq whom carbapenemase-producing E. coli or K. pneumonia were recovered, were admitted to the American University of Beirut Medical Center in July 2010. The first two patients had leukemia diagnosed upon arrival. The third patient had a complicated transurethral resection of the prostate. PCR results showed that the first isolate harboured the OXA-48 gene, the second produced both OXA-48 and NDM-1 genes, while the third isolate carried only the NDM-1 gene. They reported the first Iraqi patients referred to Lebanon from whom carbapenem resistant *Enterobacteriaceae* were recovered (El-Herte et al., 2012).

Between the years 2009 to 2011, 28 carbapenem resistant *Enterobacteriaceae* isolates were collected in the United Arab Emirates. Among these isolates 3 *Klebsiella pneumoniae*, 2 *Escherichia coli*, 1 *Enterobacter cloacae* and 1 *Citrobacter freundii* were identified to produce NDM-1 carbapenemase. These findings strongly support the assumptions that, beyond the Indian subcontinent, the Middle East is an important reservoir of NDM producing organisms (Sonnevend et al., 2013).

The first KPC outbreak outside the USA was in Israel. During 2004 and 2005, six patients were infected with carbapenem-resistant *K. pneumoniae* and admitted to the Tel Aviv Medical Center. During 2006, this number increased to reach 45 patients. Using PCR, the KPC gene was not found in any carbapenem-resistant K. pneumoniae strains in 2004. On the other hand, 93% (43 of 46) of the isolates collected from 2005 to 2006 carried the KPC gene. After sequencing, 60% of the KPC isolates identified as KPC-3 producer and the remaining were
KPC-2 genes. This research noticed a sharp increase in carbapenem-resistant K. pneumoniae strains harbouring KPC gene in Tel Aviv Medical Center from 2004 to 2006 (Leavitt et al., 2007).

KPCs in Israel considered endemic and epidemic, and many outbreaks have been reported (Nordmann et al., 2009; Leavitt et al., 2007;). In February 2005, an imipenem-resistant *E. coli* strain was isolated from a 75-year-old woman hospitalized at Tel Aviv Medical Center. During September and October 2005, three additional carbapenem-resistant *E. coli* isolates were detected. PCR confirmed the presence of the KPC-2 gene in the four isolates (Navon-Venezia et al., 2006).

Quinolone resistance is worldwide distributed. In the period from March 2003 to July 2007, a total of 141 *E. cloacae* isolates were collected from the microbiology laboratories of eight different hospitals in Algeria: five located in the centre of Algiers, two located in the east of Algiers, and the last located in the west. As a result, the qnr genes were detected only in five quinolone-resistant ESBL-producing isolates; four had the qnrS1, and one had the qnrB1. This report considered the first report for the QnrS1, QnrB1 and QnrB4 in areas such as Algeria (Iabadene et al., 2008).

### 6.16.4 Asia

The first identified acquired metallo beta-lactamases was IMP-1, it was detected in a clinical isolate of *S. marcescens* from Japan in 1994 (Osano et al., 1994). A study in Japan from April to May 1993, 105 strains of metallo beta lactamase producing Serratia marcescens were isolated from seven hospitals located in Aichi Prefecture. The results confirmed that 4 of 105 strains of *S. marcescens* tested have the IMP gene. The study researchers considered theses findings suggested a local prevalence of imipenem-resistant *S. marcescens* strains that carry the IMP gene (Ito et al., 1995).
In addition to the KPC producing isolates in USA, they have been reported from several countries outside the USA including China (Wei et al., 2007). A study carried out by Wet ZQ and his colleagues in 2004, a carbapenem-resistant K. pneumoniae strain was isolated from the sputum of a 75-year-old patient hospitalized in the ICU on November 2004. As a result, a carbapenem-resistant isolate of K. pneumoniae harbouring KPC-2 was detected in Zhejiang, China.

Another study in Singapore 2011, indicate the presence of community acquired KPC producing Enterobacteria (Venkatachalam et al., 2012).

A study carried out in China from pediatric patients characterized by a high prevalence of plasmid-mediated quinolones resistance; they found that 4.1% were positive for qnr and 8.2% for aac(6')-Ib-cr genes. They have known to confer low-level fluoroquinolone resistance or to inactivate ciprofloxacin, but not moxifloxacin (Han et al., 2010).

In Vietnam during September 2011, samples from seepage water were collected. The NDM-1 was detected in 3 Klebsiella pneumoniae isolates (Isozumi et al., 2012).

6.16.5 Latin America and Scandinavian Countries

KPC-producing isolates have now been reported from several Scandinavian countries such as a study carried out in Sweden and Norway in 2009. All the study isolates expressed a multidrug-resistant phenotype. PCR results indicated that the isolates collected from Norway harboured the KPC-2 gene while the isolates collected from Sweden harboured the KPC-3 gene. The study researchers reported this study as the first KPC-producing K. pneumoniae isolates in Norway and the second isolate from Sweden (Samuelsen et al., 2009).

KPC-producing isolates have now been reported from several countries in Latin America such as Colombia, Brazil, Trinidad and Tobago (Monteiro et al., 2009; Akpaka et al., 2009; Villegas et al., 2007). In Brazil between September 2006 and November 2006, four carbapenem-resistant K. pneumoniae strains were isolated from patients hospitalized in an
ICU of a hospital located in Recife city. These isolates were obtained from blood (2 isolates) and urine (2 isolates). PCR indicated that all the study isolates harboured the KPC gene. This study considered the first detection of KPC-2 producing K. pneumoniae strains in Brazil (Monteiro et al., 2009).

In Trinidad and Tobago 2006, a 63-yr-old male patient was admitted to a hospital in Mount Hope. As a result, the isolate was positive for the presence of carbapenemase. PCR analysis indicated the KPC gene and sequencing was performed and confirmed the KPC-2 gene (Akpaka et al., 2009).

Another study carried out in Colombia in January 2006, four isolates were collected from a tertiary care center in Medellin. Three isolates were *Pseudomonas aeruginosa* and one isolate was a *Citrobacter freundii*. As a result, the isolates carried the KPC-2 gene. It was the first report of a KPC-type beta lactamase identified not in an *Enterobacteriaceae* family (Villegas et al., 2007).

In Peru and Bolivia, a study suggested a remarkable prevalence of qnrB (54%) and, at a lower level, of qnrS (14%) in pools of commensal enterobacteria from 310 healthy children (Pallecchi L et al., 2009). In 2010, VIM-1 producing Klebsiella pneumonia isolate was detected in Denmark (Hasman et al., 2010).

Nordmann and his colleagues summarized the world wide distribution of K. Pneumoniae carbapenemase (KPC) as well as the geographic distribution of VIM and IMP genes in figures 28 and 29 (Nordmann (D) et al., 2011).
Figure 28: A) Worldwide geographic distribution of K. Pneumoniae carbapenemase (KPC) producers. Gray shading indicates regions shown separately: B) distribution in the United States; C) distribution in Europe; D) distribution in China. (Nordmann (D) et al., 2011)

Figure 29: Worldwide (A) and European (B) geographic distribution of Verona integron–encoded metallo-β-lactamase (VIM) and IMP enterobacterial producers (Nordmann (D) et al., 2011).
7 Zusammenfassung der Arbeit

Dissertation zur Erlangung des akademischen Grades

Dr.rer.med

Molecular Characterization of Carbapenemases and Quinolone Resistance Determining Region Enzymes-Producing Isolates in an Outbreak at the University Hospital of Leipzig

eingereicht von:

Hala Alqasem

angefertigt am Institut für Medizinische Mikrobiologie und Infektionsepidemiologie/

Universitätsklinikum Leipzig

Betreut von: Prof. Dr. med. Arne Rodloff

30. Juli 2014

Beta lactam resistance producing isolates of Enterobacteriacea and non-Enterobacteriacea have emerged since more than seventy years ago (Abraham and Chain, 1940). They are known to cause both community and hospital-acquired infections. Resistance against carbapenem is primarily mediated by the production of enzymes that destroy the beta lactam antimicrobials, which are produced by these isolates involving the expression of serine and metalobetalactamase genes KPC, IMP, VIM, NDM-1 and OXA-48. Quinolone resistance is predominantly mediated by mutations in the qnrA, qnrB, qnrS, and aac-6-Ib genes. Carbapenemase-producing organisms especially Klebsiella pneumoniae carbapenemases (KPCs) emerged as important pathogens especially among critically ill patients causing significant morbidity and mortality. This study aims to determine the prevalence and types of quinolone resistance and carbapenemases genes among different isolates from patients admitted to the University Hospital of Leipzig over a period of ten months. During the period from January 2011 through October 2011, a total of 50 carbapenemases isolates were recovered from patients of the University Hospital of Leipzig/ Germany. The
isolates were identified by biochemical tests and their susceptibility to antimicrobials was determined by the microbroth dilution method according to ISO standard. The KPC, IMP, VIM, OXA-48, NDM-1, and aac-6-Ib genes as well as qnrA, qnrB, and qnrS genes were detected by multiplex PCR, respectively.

Results showed that KPC gene was detected in 82% of the isolates while 8% were KPC negative. The qnrA, qnrS, IMP, NDM-1, and OXA-48 genes were not detected in any of the isolates while qnrB and VIM genes were found in 2%. On the other hand, aac-6-Ib gene was the most prevalent gene among the study isolates and composed a percentage of 96%. Results also showed that KPC, and aac-6-Ib genes were detected in isolates collected from urine, blood, wounds, swabs, sputum, tracheal secretions, biopsies, and anal smears, while VIM gene was detected in one isolate collected from blood. The qnrB gene was found in one isolate collected from urine specimen.

The wide spread of carbapenem and quinolone resistance-producing organisms is a critical problem that complicates the treatment of infections resulting from these organisms. Necessary measures must, therefore, be taken to limit their spread, which include appropriate antibiotic treatment, control of hospital infections, observe of personal hygiene, and the use of appropriate methods of sterilization and disinfection to prevent the dissemination of these organisms.

**Keywords:** Resistance, carbapenemases, QRDR, multiplex PCR, antimicrobials
8 Conclusion

Based on results of this study, *Klebsiella pneumoniae* carbapenemase gene and aac-6-Ib gene of Quinolone resistance determining region were the major genes found in strains of an outbreak at the University Hospital of Leipzig being responsible for a variety of serious infections. The main organisms that were found to be responsible for the infections were mainly *Klebsiella pneumoniae* followed by *E. coli* and *Enterobacter cloacae*, then *Acinetobacter baumannii* and *Proteus mirabilis*. Sources from where the isolates were collected were urine, followed by blood, then swabs, and finally wounds. The KPC gene was mainly found in organisms collected from urine, followed by wounds, and finally blood. While qnrB, VIM genes were found in one isolate collected from urine and blood, respectively. The most prevalent quinolone resistance determining region gene was aac-6-Ib and it was found in 100% of the isolates collected from blood, wounds, sputum, tracheal secretions, biopsies, and anal smears, followed by urine 94% and then swabs 89%. On the otherhand, qnrA, qnrS, IMP, OXA-48, and NDM-1 genes were not found in any of the isolates.

The median age of infection with carbapenemase /QRDR producing organisms was 53 years and males were more likely to be infected than females. Results showed a significant relationship between infections caused by carbapenemase and/or QRDR producing organisms and the weather time, infections increased mainly in winter time with lower temperature and decreased in summer.
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has a different evolutionary origin from that of β-lactamases of the penicillinase type.

101. Jawahar Swaminathan and MSD staff at the European Bioinformatics Institute.

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http://www.ebi.ac.uk/pdbe-srv/view/entry/1bsg/summary

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## 10 Appendices:

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<td>The Same Pattern</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>K. Pneumoniae</td>
</tr>
<tr>
<td>49</td>
<td>VA2360</td>
<td>Alona</td>
<td>1960</td>
<td>Wound secretions abdomen</td>
<td>31.01.2011</td>
<td>N</td>
<td>Different Pattern</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>E. coli</td>
</tr>
<tr>
<td>50</td>
<td>VA3873</td>
<td>XXXX</td>
<td>XXXX</td>
<td>Swabs</td>
<td>16.02.2011</td>
<td>N</td>
<td>Different Pattern</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>P</td>
<td>K. Pneumoniae</td>
</tr>
</tbody>
</table>

SUM | Samples | 41 P | Same Pattern | 0 | 1 | 0 | 1 | 0 | 0 | 0 | 48 |

App. 1: The Study isolates summary table
11 Erklärung über die eigenständige Abfassung der Arbeit


29.07.2014
Datum

........................................
Unterschrift
Curriculum Vitae

PERSONAL

Full Name: Hala Burhan Al-Qasem
Nationality: Jordanian
Languages: Arabic; mother tongue
English; very good
French; intermediate
German; basics

Date of Birth: 12.03.1984
Place of Birth: Amman
Profession: Medical Laboratory Sciences

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EDUCATION

10/2011 - 07/2014 University of Leipzig
PhD Candidate in Medical Microbiology
Thesis Title: Molecular Characterization of Carbapenemases and Quinolone Resistance Determining Region Enzymes-Producing Isolates in an Outbreak at the University Hospital of Leipzig

Master Degree of Medical Laboratory Sciences
Thesis Title:
“Molecular characterization of extended-spectrum beta lactamase-producing \textit{E. coli} and \textit{Klebsiella} species isolates at the Jordan University Hospital and King Hussein Cancer Center”

Short Running Title:
“Molecular characterization of ESBL-producing \textit{E. coli} and \textit{Klebsiella} species isolates”

10/2002 - 06/2006 Jordan University of Science and Technology (J.U.S.T), Irbid-Jordan
Bachelor Degree of Medical Laboratory Sciences

General Certificate of Secondary Education (GCSE)-Scientific Stream, 88.9% GPA
WORK EXPERIENCE

4/ 2009-8/2010 Jordanian Royal Medical Services; King Hussien Medical City Amman-Jordan
Rotation at the hospital laboratory departments:
- Blood Bank
- Clinical Chemistry and Hormones
- Haematology
- Immunology
- Microbiology

TRAINING COURSES

04 and 11.02/2011 Technical University Bergakademie Freiberg, Freiberg-Germany - Communication and Presentation Skills course
02/ 2007-03/ 2007 University of Jordan, Amman-Jordan - In-vitro Fertilization course
04/ 2007-05/ 2007 Jordan University Hospital, Amman-Jordan - In-vitro Fertilization Training course
09/ 2006- 04/ 2007 Al Khaldi Hospital, Amman-Jordan - Practical training at the hospital laboratory departments
09/ 2005- 06/ 2006 Al Bashir Hospital and King Hussein Medical City, Amman-Jordan - Co-op Trainee, Practical Training at the hospital laboratory departments

LANGUAGE COURSES

05/ 2010-07/2010 Goethe Institute, Amman-Jordan, - German Language: Grundstufe 1
08/ 2008-07/ 2009 Le Centre Culturel Français d'Amma-Jordan - French Language Courses
02/ 2004- 04/ 2004 The American Language Centre, American Embassy Amman-Jordan - English Language Course: Listening and Speaking
06-07/ 1999 The British Council, Amman-Jordan - English Language Course: TI 1A-Y
RESEARCH AREAS OF INTEREST

- Molecular Microbiology
- Understanding host-pathogen interactions
- In-vitro Fertilization analysis

PROFESSIONAL COMPUTER SKILLS

- Microsoft Office
- Graphic Design
- Adobe Photoshop
- Statistical Analysis (SPSS 16)
Acknowledgment

First, I would like to express my sincere gratitude to my supervisor Prof. Arne Rodloff who gave me the honor to work under his supervision and for supporting my PhD study and research. He patiently provided insightful comments, suggestions, and the advice necessary to complete my doctoral project. I gained from his great scientific experience a lot. I highly appreciated his sense of developing my thesis day by day. For every thing you have done for me, Prof. Rodloff, I thank you.

I want to express my gratitude to the medical microbiology and infections epidemiology laboratory of Leipzig University Hospital for providing the isolates. I would like to thank Prof. König for supporting me and solving any technical problems appeared during the laboratory experiments. My deepest thank is for Mrs. Hennig-Rolle for her kindness, friendship, help, and support. She gave me the time for any enquiry; provided me with any missing materials, and information. I am most grateful to Mrs. Pöschel for providing me with the laboratory materials, working place, and the results of the gel electrophoresis test for the research isolates. I would like to express the deepest appreciation to Mrs. Wojcik for her kind communication, friendship, help, and support during my research time. She is a real friend who is always available.

From other universities I would especially like to thank Prof. Soeren Gatermann from Ruhr University of Bochum for providing me with the aac-6’-Ib strains, Prof. Yvonne Pfeifer from Robert-Koch Institut for providing OXA-48 and NDM-1 strains, and Prof. Daniel Jonas from the Medical Center of the University of Freiburg for providing qnrA, qnrB, and qnrS strains. All were used as positive controls during this my PhD research.

Finally, I would like to thank everyone who helped me during my study period and I did not thank him by his name. I also thank all others who were always willing to help.