Phenotypic and Molecular Detection of *Klebsiella pneumonia* Carbapenemase in *Enterobacteriaceae*

Thesis

Submitted for Partial Fulfillment of Master Degree
in **Clinical and Chemical Pathology**

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2014


وأنزل الله عليك
الكتاب والحكمة
وعلَّمَكَ مَا لَمْ تَكُن
تَعْلَمُ وكَانَ فَضْلُ
اللَّهِ عَلَيْكَ عَظِيماً
صدق الله العظيم
سورة النساء الآية (١١٣)

Acknowledgment

First and foremost, I feel always indebted to ***ALLAH****, the Most Merciful, Who gives me power to accomplish this work.*

I would like to express my deepest appreciation and sincere gratitude to ***Professor/ Nevine Nabil Kassem,*** *Professor of Clinical and Chemical Pathology, Faculty of Medicine – Ain Shams University, for her sincere help, constant encouragement, constructive criticism, and valuable guidance; I was truly honoured to work under her supervision.*

I wish also to express my great gratitude and utmost appreciation to ***Doctor/ Samia Abdou Girgis,*** *Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine – Ain Shams University for her valuable suggestions and instructions, the effort and time she has devoted to the fulfillment of this work.*

I feel deeply indebted to ***Doctor/ Hala Badr El-Din Ali,*** *Assistant Professor of Clinical and Chemical Pathology, Faculty of Medicine – Ain Shams University, for her active cooperation, deep concern and enthusiastic encouragement during the progress of this work.*

I owe special thanks to ***my Family*** *for their care, patience and continuous encouragement.*

Shereen Mahmoud Abdou

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List of Abbreviations

Abb.	Full item
ABC	Adenosine triphosphate (ATP)-binding cassette
AcrAB	Acriflavine resistance protein A and B
AK	Amikacin
ALG	Alginate
AMC	Amoxicillin- clavulanic acid
AmpC	Ambler Class C
APB	aminophenyl-boronic acid
BA-CD	Boronic acid combined disc test
bla	Beta -lactamase
BMD	Broth microdilution
CAZ	Ceftazidime
CDC	Center for Disease Control and Prevention
CFP	Cefoperazone
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards Institute
CM	Cytoplasmic membrane
CP	Carbapenemase-producing
CPD	Cefpodoxime
CRO	Ceftriaxone
CTX	Cefotaxime
CTX-Ms	Cefotaximase
ddNTP	dideoxynucleotide triphosphate
DDST	Double disc synergy test
E. coli	*Escherichia coli*
E-test	Epsilometer test
EDTA	Ethylene-diamine-tetra-acetic acid
ESBLs	Extended-spectrum β- lactamases
EU	European Union
F	Nitrofurantoin
FEP	Cefrpime
FOX	Cefoxitin
GES	Guiana extended spectrum beta- lactamase
GIcNAc	N-acetylglucosamine
GIM	German imipenemase
HEPES	N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid
IBC	Integron borne cephalosporinase
ICU	Intensive care unit
IEF	Isoelectric focusing

Abb.	Full item
IM	inner membrane
IMP	Inner membrane proteins
IND	indologenes
IPM	Imipenem
K.	*Klebsiella*
KPC	*Klebsiella pneumoniae* carbapenemase
LPS	Lipopolysaccharide
LTA	Lipoteichoic acid
MATE	Multidrug and toxic compound extrusion
MBLs	Metallo-ß-lactamases
MB-PCR	molecular beacons-polymerase chain reaction
MDR	Multi drug resistant
MEM	Meropenem
MFP	membrane fusion protein
MFS	Major facilitator superfamily
MHT	Modified Hodge Test
MIC	Minimum inhibitory concentration
MRSA	Methicillin resistant *Staphylococcus aureus*
MurNAc	N-acetylmuramic acid
NAG	N-acetylglucosamine
NAM	N-acetylmuramic acid
NDM	New Delhi metallo-ß-lactamase
NMC	Non metalloenzyme carbapenemase
OM	Outer membrane
OMP	Outer membrane proteins
OmpC	Outer membrane protein C
OmpF	Outer membrane protein F
Omp K35	Osmoporins of *klebsiella pneumoniae*
OXA	Oxacillin
OXA-MHT	Oxacillin –Modified Hodge Test
P.	*Pseudomonas*
PBPs	Penicillin Binding Proteins
PC1	penicillinases 1
PCR	Polymerase Chain Reaction
PFGE	Pulsed- Field Gel Electrophoresis
PG	*Peptidoglycan*
pIs	Isoelecteric points
Qnr	quinolones resistance
RND	*Resistance-nodulation-cell-division*
S.	*Streptococcus*

Abb.	Full item
S. marcescens	*Serratia marcescens*
SHV	s*u*lf*h*ydryl *v*ariable
SIM	Seoul imipenemase
SME	*Serratia marcescens* enzyme
SMR	Small multidrug resistance
SPM	San Paulo metallo-β-lactamase
Spp.	Species
SXT	Trimethoprim-Sulfamethoxazole
TEM	Temoneira (name after the patient providing the first sample)
TZP	Tazobactam
US	United States
UTI	Urinary tract infection
VIM	Verona integron-encoded metallo-β-lactamase
Zn	Zinc

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Introduction

Carbapenems are commonly used to treat life-threatening infections caused by multidrug-resistant *Enterobacteriaceae*; these drugs represent the last line of defense against serious or invasive infection ***(Pillai et al., 2009).*** During the last decade, Carbapanem resistance has emerged among clinical isolates of the *Enterobacteriaceae* family, and this is increasingly attributed to the production of the β-lactamases capable of hydrolyzing carbapenems. Among those enzymes, a new type of Ambler class A β-lactamase, the *Klebsiella pneumoniae* Carbapenemaze (KPC), which has been rapidly spreading among K. pneumoniae isolates and other *Enterobacteriaceae* ***(Tsakris et al., 2009b).***

Treatment of infection caused by KPC bacteria is particularly worrisome as the carbapenems are often agents of the last resort for resistant Gram-negative infections. The optimal treatment of infections caused by KPC bacteria is not well established and clinical outcome data remain sparse ***(Hirsch and Tam, 2010).*** So the rapid global spread of *Klebsiella pneumoniae* that produces *K.pneumoniae* carbapenemase (KPC), is of major concern ***(Pillai et al., 2009).***

Laboratory diagnostic methods for *blaKPC* include susceptibility testing for meropenem, imipenem, and ertapenem which are determined by broth microdilution, disk diffusion, E-test, Microscan, and the Vitek 2 test; also phenotypic methods as modified Hodge test could be used. Similarly, CHROM agar KPC has shown good sensitivity and specificity for screening ***(Lolans et al., 2010).***

Phenotypic methods utilizing boronic acid disc tests have demonstrated promising results and appear practical for use in clinical microbiology laboratories ***(Hirsch and Tam, 2010)***. Boronic acid compounds are serine-type B-lactamase inhibitors that were employed originally for the detection of class C plasmidic AmpCs in *Enterobacteroaceae*. Recently, they have also been evaluated for the differentiation of KPC-producing *Enterobacteriaceae.* In that respect, combined-disc tests using carbapenems with and without phenylboronic acid (PBA) have been proposed as the most accurate phenotypic tests for detecting KPC production ***(Pournaras et al., 2010*)**.

The modified hodge test was also evaluated for detection of KPC-mediated resistance. This is a phenotypic test which could be used to determine if reduced susceptibility to carbapenems is mediated by a carbapenemase. The test is demonstrated 100% sensitivity and specificity for detection of KPC activity ***(Doi et al., 2008).***

The gene encoding the KPC enzyme is usually flanked by transposon-related sequences and has been identified on conjugative plasmids; therefore, the potential for dissemination is significant. Isolates that acquired this enzyme are usually resistant to several other classes of antimicrobial agents used as treatment options. So accurate laboratory identification of KPC-producing clinical isolates will be critical for limiting the spread of this resistance mechanism. Nucleic acid detection methods as the real-time, rapid-cycle PCR testing are fast, accurate means of identifying *blaKPC* ***(Anderson et al., 2007).***