A New Phenotypic Method for Detection of Extended Spectrum β-lactamases in AmpC β-lactamase -producing *Klebsiella Species*

Thesis

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By

Marwa Ghaleb Hamdy Mohamed Abd el Hamid
MB BCh. Ain Shams University

Under Supervision of

Doctor / Hala Badr El-Din Ali Othman
Assistant Professor of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Doctor/ Dalia Hosni Abd El-Hamid Ahmed
Lecturer of Clinical and Chemical Pathology
Faculty of Medicine, Ain Shams University

Faculty of Medicine
Ain Shams University
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Dedication

I would like to dedicate this thesis

To the soul of my Father, to my Mother and to my

beloved husband

to them I will never find adequate words

to express my gratitude.

Also to all my Family for dealing tactfully

and patiently during this work.

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LIST OF ABBREVIATIONS

Abb.	Meaning
AMC	Amoxicillin/clavulanate
ASC	Active surveillance cultures
ATM	Aztreonam
BA	Boronic acid
BD Phoenix	Becton Dickinson Phoenix Automated Microbiology System
BES-1	Brazilian ESBLs
BIL	Bilal
BZBTH2B	Benzo (β) thiophene-2-boronic acid
C. amalonaticus	*Citrobacter amalonaticus*
C.freundii	*Citrobacter freundii*
CAZ	Ceftazidime
CDC	Centers for Disease Control and Prevention
CFU	Colony forming unit
CIAT	Ceftazidime-imipenem antagonism test
CLOX	Cloxacillin
CLSI	Clinical and Laboratory Standards Institute
CMY	Cephamycins
CPD	Cefpodoxime
CRO	Ceftriaxone
CTT	Cefotetan
CTX	Cefotaxime
CTX-M	Cefotaximase-Munich
ddATP	Dideoxynucleotide adenosine triphosphate
ddCTP	Dideoxynucleotide cytosine triphosphate
ddGTP	Dideoxynucleotide guanine triphosphate
DDM	Disc diffusion method
DDNTP	Dideoxynucleotide triphosphate
DDST	Double-disc synergy test

LIST OF ABBREVIATIONS *(Cont...)*

Abb.	Meaning
ddTTP	Dideoxynucleotide thiamine triphosphate
DHA	Dhamam, Saudi Arabia
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonuclic acid
DSDT	Double Synergy Differential Test
DTDT	Direct Three-dimensional test
E- Test	Epsilometer test
E. aerogenes	*Enterobacter aerogenes*
ESAC	Extended spectrum AmpC
ESBLs	Extended spectrum β-lactamase
EUCAST	European committee on antimicrobial susceptibility testing
FOX	Cefoxitin
GES	Guyana ESBLs
hrs	Hours
IEF	Isoelectric focusing
IRT	Inhibitor-resistant TEM
ITDT	Indirect Three-dimensional test
K.Oxytoca	*Klebsiella Oxytoca*
LAT	Latamoxef
LCR	Ligase chain reaction
M. morganii	*Morganella morganii*
M3D	Modified three dimensional test
MBL	Metallo beta lactamase
MDDST	Modified double-disc synergy test
MDRO	Multi drug resistance organisms
MHA	Mueller-Hinton agar
MIC	Minimal inhibitory cocentration
MOX	Moxalactam
ND	Non determinable
P. aeruginosa	*Pseudomonas aeruginosa*

P. mirabilis ..... *Proteus mirabilis*

LIST OF ABBREVIATIONS *(Cont...)*

Abb.	Meaning
PCDDT	Phenotypic confirmatory disc diffusion test
PCR RFLP	PCR restriction fragment length polymorphism analysis
PCR	Polymerase chain reaction
PCR-SSCP	PCR-single-strand conformation polymorphism
PER	*Pseudomonas* extended resistance
PI	Isoelectric point
S. marcescens	*Serratia marcescens*
S. pneumonia	*Streptcocci pneumonia*
S.enterica	*Serratia enterica*
SFO	*Serratia fonticola*
SHV	Sulfhydryl variable
spp	Species
TDT	Three-dimensional test
TEM	Temoniera
TLA	Tlahuicas (Indian tribe)
Tris-EDTA	Tris ethylene diamine tetra acetic acid
TZP	Tazopactam
U.K	United Kingdom
U.V. light	Ultra violet light
UTI	Urinary tract infection
VEB	Vietnam extended-spectrum β-lactamase

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INTRODUCTION

Increasing prevalence of multidrug-resistant Gram-negative bacteria has continuously been reported over the past years, in particular *Enterobacteriaceae* producing extended spectrum β-lactamases (ESBLs). ESBLs have the ability to hydrolyse penicillins, first- second- and third-generation cephalosporins and aztreonam (but not cephamycins or carbapenems), and their activity is decreased by inhibitors such as clavulanic acid. ESBL-producing organisms may be responsible for life-threatening infections, leading to increased morbidity, mortality and healthcare-associated costs challenge ***(Polsfuss et al., 2012).***

Ambler class C β-lactamases (AmpCs) have gained importance since the late 1970s as one of the mediators of antimicrobial resistance in gram negative bacilli. These enzymes are cephalosporinases capable of hydrolyzing all β-lactams to some extent. AmpCs are two types, plasmid-mediated and chromosomal or inducible AmpC. Chromosomal AmpC enzymes are seen in organisms such as *Citrobacter freundii*, *Enterobacter cloaca*, *Morganella morganii*, *Hafnia alvei* and *Serratia marcescens* and are typically inducible by β-lactam antibiotics such as cefoxitin and imipenem but poorly induced by the third or fourth generation cephalosporins ***(Akujobi et al., 2012)***.

The absence of new effective anti-gram-negative antibiotics makes infection control the most important counter measure against multidrug-resistant gram-negative pathogens. Infection control can prevent additional infections and the spread of resistant pathogens and thereby reduce the need to use antibiotics. Infection control is most effective when directed by rapid, accurate laboratory results ***(Thomson, 2010)***.

In recent years, the prevalence of infections with multidrug resistant *Enterobacteriaceae* has steadily increased. *Entero-bacteriaceae* producing AmpCs have become a major therapeutic challenge ***(Polsfuss et al., 2011)***.

There are numerous reports in which *Klebsiella pneumonae* and *Escherichia coli(E. coli)* isolates have been found to produce both plasmid-mediated AmpC β-lactamases (PMABLs) and ESBLs ***(Song et al., 2006)***.

The inhibitor-based confirmatory test approach is most promising for isolates that do not co-produce an inhibitor-resistant β-lactamase like AmpC. However, a high-level production of AmpC may prevent the detection of an ESBL. Moreover, in these organisms, clavulinic acid may act as an inducer of high-level AmpC production resulting in an increase in the resistance of the isolate to other screening drugs, producing a false-negative result in the ESBL detection test. Therefore, there is a need for alternative method that detects ESBL in *Klebsiella spp*. and *E. coli* isolates with high

sensitivity even though the isolates harbor plasmid AmpC ***(Khan et al., 2008)***.

Now, modified double disc synergy test (MDDST) can be used. Which differs from the original one in two respects: first is the use of cefepime (4th generation cephalosporins) as an indicator drug which is known to be a poor substrate for AmpC B-lactamases making this drug a more reliable agent for ESBL detection in the presence of an AmpC enzyme ***(Khan et al., 2008),*** second is the use of Tazopactam instead of clavulanic acid as the later may act as an inducer of high level AmpC producing a false negative result in the ESBL elective test while Tazopactam and sulbactam are much less likely to induce AmpCs ***(Pitout et al., 2003)***.