

# **A New Phenotypic Method for Detection of Extended Spectrum $\beta$ -lactamases in AmpC $\beta$ -lactamase -producing *Klebsiella Species***

*Thesis*

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*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  
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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 ( $\beta$ ) thiophene-2-boronic acid
<i>C. amalonaticus</i> .....	<i>Citrobacter amalonaticus</i>
<i>C. freundii</i> .....	<i>Citrobacter freundii</i>
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 .....	Cephameycins
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.....	Deoxyribonucleic acid
DSDT .....	Double Synergy Differential Test
DTDT .....	Direct Three-dimensional test
E- Test .....	Epsilometer test
<i>E. aerogenes</i> .....	<i>Enterobacter aerogenes</i>
ESAC .....	Extended spectrum AmpC
ESBLs .....	Extended spectrum $\beta$ -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
<i>K.Oxytoca</i> .....	<i>Klebsiella Oxytoca</i>
LAT .....	Latamoxef
LCR.....	Ligase chain reaction
<i>M. morganii</i> .....	<i>Morganella morganii</i>
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 concentration
MOX.....	Moxalactam
ND.....	Non determinable
<i>P. aeruginosa</i> .....	<i>Pseudomonas aeruginosa</i>

*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.....	<i>Pseudomonas</i> extended resistance
PI.....	Isoelectric point
<i>S. marcescens</i> .....	<i>Serratia marcescens</i>
<i>S. pneumonia</i> .....	<i>Streptococci pneumonia</i>
<i>S. enterica</i> .....	<i>Serratia enterica</i>
SFO .....	<i>Serratia fonticola</i>
SHV.....	Sulphydryl 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 $\beta$ -lactamase

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## INTRODUCTION

**I**ncreasing prevalence of multidrug-resistant Gram-negative bacteria has continuously been reported over the past years, in particular *Enterobacteriaceae* producing extended spectrum  $\beta$ -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  $\beta$ -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  $\beta$ -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  $\beta$ -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 pneumoniae* and *Escherichia coli*(*E. coli*) isolates have been found to produce both plasmid-mediated AmpC  $\beta$ -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  $\beta$ -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*).