Phenotypic-detection of Plasmid-mediated AmpC β-Lactamase-producing Strains of Proteus Mirabilis

Thesis

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List of Abbreviations

ABC	: ATP-binding cassette
ACC	: Ambler class C
AcrAB	: Acriflavine resistance protein A and B
ACT	: AmpC type
Ala	: Alanins residue
ALG	: Alginate
AmpC	: Ambler class C enzymes
AP-PCR	: Arbitrarily primed polymerase chain reaction
BAs	: Boronic acids
BIL-1	: Bilal
CA	: Clavulanic acid
CAM	: Cefoxitin agar medium
CAUTI	: Catheter associated urinary tract infection
CAZ	: Ceftazidime
CIAT	: Ceftazidime-imipenem antagonism test
CLSI	: Clinical and laboratory standards institute
CM	: Cytoplasmic membrane
CMY	: Cephalosporins mediated by β-lactamas
CMY	: Cephamycins
CTT	: Cefotetan
CTX	: Cefotaximase
DDST	: Double-disk synergy test
DHA	: Dhahran
E.coli	: *Escherichia coli*
ECA	: Enterobacterial common antigen
EDTA	: Ethylene-diamine-tetra-acetic acid
ESAC	: Extended-spectrum AmpC
ESBL	: Extended-spectrum β-lactamase
E-test	: Epsilometer test
FOX	: Cefoxitin
IEF	: Isoelectric focusing
IgA	: Immunoglobulin A
IM	: Inner membrane
IMP	: Inner membrane protein
IMPs	: Imipenem hydrolyzing enzymes
IPM	: Imipenem
K.pneumoniae	: Klebsiella pneumoniae
KPC	: Klebsiella pneumonia carbapenemase

List of Abbreviations *(Cont...)*

LAT	: Latamoxef
LPS	: Lipopolysaccharide
MATE	: Multidrug and toxic compound extrusion
MBLs	: Metallo β-lactamases
MBLs	: Metallo β-lactamases
MDDM	: Modified double disk approximation method
MDR	: Multi drug resistant
MFP	: Membrane fusion protein
MFS	: Major facilitator superfamily
MIC	: Minimal inhibitory concentration
MIR-1	: Miriam
MOX	: Moxalactam
MRSA	: Methicillin resistant Staphylococcus aureus
NAG	: N-acetylglucosamine
NAM	: N-acetylmuramic acid
NCCLS	: National committee for clinical laboratory standards
NMC-A	: Non-metallo carbapenamase of class A
OM	: Outer membrane
OMP	: Outer membrane protein
OMPF	: Outer membrane protein F
OXA	: Oxacillinase
P.mirabilis	: *Proteus mirabilis*
PABLs	: Plasmid mediated AmpC β-lactamases
PBPs	: Penicillin binding proteins
PC1	: Penicillinases
PCR	: Polymerase chain reaction
PFGE	: Pulsed field gel electrophoresis
PG	: Peptidoglycan
PL	: Phospholipid
RA	: Rheumatoid arthritis
RND	: Resistance nodulation cell division
SHV	: Sulfhydryl *v*ariable
SMR	: Small multidrug resistance
Spp.	: Species
TDET	: Three dimentional extract test
TE	: Tris EDTA
TEM	: Temoneira
UTI	: Urinary tract infections
VIM	: Verona integron encoded metallo-β-lactamase

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INTRODUCTION

Proteus mirabilis which belongs to *Enterobacteriaceae* family, is part of the normal flora of the human gastrointestinal tract. It is the second most common cause of urinary tract infections and one of the important causes of nosocomial infections ***(Song et al., 2011).*** It produces infections in humans only when the bacteria leave the intestinal tract. They produce bacteremia, pneumonia, and focal lesions in debilitated patients or those receiving intravenous infusions ***(Jawetz et al., 2007).***

β-Lactamase-mediated resistance to penicillins and cephalosporins is a significant problem among gram-negative bacteria worldwide. β-Lactamases can be divided into four major classes (A, B, C, and D) based on substrate profiles and amino acid sequence ***(Tenover et al., 2009).***

AmpC β-lactamases 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. AmpC β-lactamases 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 countermeasure 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. *Enterobacteriaceae* producing AmpC β-lactamases (AmpCs) have become a major therapeutic challenge ***(Polsfuss et al., 2011).***

P.mirabilis is often inhibited by penicillins. Oxyimino-cephalosporins have been used as the drugs of choice to treat infections caused by ampicillin-resistant *P.mirabilis*. However, as with other *Enterobacteriaceae*, *P. mirabilis* strains exhibiting resistance to expanded-spectrum β-lactam agents have been widely reported in many parts of the world ***(Song et al., 2011).***

The detection of AmpC-producing *P. mirabilis* is of significant clinical relevance since AmpC producers may appear susceptible to expanded-spectrum cephalosporins when initially tested. This may lead to inappropriate antimicrobial regimens and therapeutic failure. Thus, a simple and reliable detection procedure for AmpC producers is needed ***(Polsfuss et al., 2011).***

It may be difficult to detect plasmid-mediated AmpC β-lactamases (PABLs), which are known to interfere with the therapeutic and infection control processes ***(Lee et al., 2009).***

Many methods for the detection of ESBLs and PABLs have been proposed, but some procedures are difficult to perform in practice, time-consuming, and hard to interpret ***(Jeong et al., 2009).***

Phenotypic detection methods can be divided into the following two categories: ones that detect AmpC activities in enzyme extracts and the others that evaluate the inhibitory effects induced by AmpC inhibitors. Three dimensional extraction test has been reported as a reliable enzyme-extraction method, but this method is complicated and not applicable to clinical microbiology laboratories. Cephamycin-Hodge test and Tris-EDTA (TE)-disk test are reported as simple and sensitive phenotypic detection methods ***(Lee et al., 2009).***

Boronic acid (BA) derivatives are reported as reversible inhibitors of AmpC enzymes. Many studies have validated the use of BA to detect AmpC β-lactamases among gram-negative bacteria ***(Shoorashetty et al., 2011).***

Because phenotypic tests do not differentiate between chromosomal and plasmid -mediated AmpC β-lactamases, plasmid-mediated AmpC β-lactamases are most accurately detected with multiplex AmpC PCR. If molecular testing is not available, screen-positive isolates should be tested with a

phenotypic confirmatory test that will distinguish AmpC production from other resistance mechanisms. Phenotypic confirmatory tests based on the detection of cephamycin hydrolysis or AmpC inhibition will distinguish AmpC β-lactamases from ESBLs and porin mutation. Confirmatory tests that detect cephamycin hydrolysis include the AmpC disk test, modified Hodge test, and the three-dimensional test. These are performed separately from the routine susceptibility test ***(Thomson, 2010).***