Phenotypic and Genotypic Detection of Carbapenemase Producing *Pseudomonas aeruginosa* Recovered from Clinical Specimens

Thesis

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Contents

List of Abbreviations i

List of Tables v

List of Figures vii

Introduction 1

Aim of the Work 4

Review of Literature

- Carbapenemases 5
- Laboratory Detection of Carbapenemase Producing *Pseudomonas aeruginosa* 13
- Treatment of Carbapenemase Producing *Pseudomonas aeruginosa* 48
- Infection Control Strategies........ 59

Materials and Methods 66

Master Sheet........ 84

Results........ 87

Discussion 98

Summary 110

Conclusion and Recommendations 113

References........ 115

Arabic summary .... --

List of Abbreviations

Abb.	Meaning
2-MPA	2- mercaptopropionic acid
A. baumannii	Acinetobacter baumannii
ABS	Antimicrobial Stewardship
ACHN-490	Plazomicin
AHLs	N-acylated homoserine lactone regulatory system
AIM	Adelaide imipenmase
AmpC	Ambler Class C
APB	3-aminophenylboronic acid
BAL 30072	Siderophore monosulfactam
BCII	Metallo-β-lactamase from *Bacillus cereus*
bla	Beta –lactamase gene
BlaB or GOB-1	Metallo-β-lactamase from *Chryseobacterium meningosepticum*
C. freundii	*Citrobacter freundii*
Ca2+	Calcium
CDT	Combined disc test
CFU	Colony forming unit
CHDL	Carbapenem-hydrolyzing class D β-lactamases
CLSI	Clinical Laboratory Standard Institute
CP	Contact precautions
CPE	Carbapenemase-producing *Enterobacteriacae*.
CPGN	Carbapenemase-producing Gram-negative bacilli
CphA	Metallo-β-lactamase from *Aeromonas hydrophilia*
CRE	Carbapenem-resistant *Enterobacteriaceae*
CRPA	Carbapenem resistant *P. aeruginosa*
CTX-Ms	Cefotaximase
DDST	Double disc synergy test
DIM	Dutch imipenrmase
DNA	Deoxy ribonucleic acid
DOR	Doripenem
DPA	Dipicolinic acid
E. cloacae	*Enterobacter cloacae*

List of Abbreviations

Abb.	Meaning
E. coli	*Escherichia coli*
EC	Environmental cleaning
EDTA	Ethylene diamine tetra-acetic acid
ESBL	Extended spectrum β-lactamases
E-test	Epsilometer test
ETP	Ertapenem
FEZ-1	Metallo-β-lactamase from *Legionella gormannii*
FIM	Florence imipenemase
GES	Guiana extended spectrum
GIM	German imipenemase.
GNB	Gram-negative bacilli
HCWs	Health care workers
HH	Hand hygiene
ICU`	Intensive care unit
IEF	Isoelectric focusing
IMP	Imipenem-hydrolyzing β-lactamases
IND-1	Metallo-β-lactamase from *Chryseobacterium indologenes*
IPM	Imipenem
JOHN-1	Metallo-β-lactamase from *Flavobacterium johnsoniae*
K. pneumonia	*Klebsiella pneumoniae*
***KESC* group**	*Klebsiella, Enterobacter, Serratia, Citrobacter*
KPC	Klebsiella pneumoniae carbapenemases
L1	Metallo-β-lactamase from *S. maltophilia*
MALDI-TOF	Matrix-assisted laser desorption ionization-time of flight
MBL	Metallo-β-lactamases
Mbl1B	Metallo-β-lactamase from *Caulobacter crescentus*
MDR	Multidrug resistant
MDRPA	Multidrug resistant *Pseudomonas aeruginosa*
ME1071, CP3242	Maleic acid derivative

List of Abbreviations

Abb.	Meaning
MEM	Meropenem
Mex	Multidrug efflux
Mg2+	Magnisium
MHA	Muller Hinton agar
MHT	Modified Hodge test
MIC	Minimal inhibitory concentration
MK-7655	Novel β-lactamase inhibitor
MS	Mass spectrometry
NAB 7061	Polymyxin derivative
NAB 739	Polymyxin derivative
NDM	New Delhi metallo-β lactamase
NMC	Non metalloenzyme carbapenemases
NXL104	Avibactam
Opr	Outer membrane porin
OXA	Oxacillinase
P.aeruginosa	*Pseudomonas aeruginosa*
PAE-MHT	*Pseudomonas aeruginosa*- modified Hodge test
PCR	Polymerase Chain Reaction
PFGE	Pulsed field gel electrophoresis
QS	Quorum-sensing system
QSI	Quorum-sensing inhibitors
RCS	Rapid CARB screen test
SFH-1	Metallo-β-lactamase from *S. fonticola*
SHV	Sulfhydryl variable
SIM	Seoul imipenemase
SMA	Sodium mercaptoacetic acid
SmaI	Restriction enzyme for PFGE
SPM	Sao Paulo metallo-β-lactamase
ß-GAL	ß-galactosidase
ß-GUR	ß-glucuronidase
TDA	Tryptophan deaminase reaction
THIN-B	Metallo-β-lactamase from *Janthinobacterium lividium*
TUS-1, MUS-1	Metallo-β-lactamase from *Myroides spp.*

List of Abbreviations

Abb.	Meaning
VIM	Verona integron-encoded metallo-β-lactamases
XbaI	Restriction enzyme for PFGE
ZI	inhibition zone
Zn^{2+}	Zinc
$ZnSO_4$,	Zinc sulphate
β-Lactamase	beta-lactamase

List of Tables

Table No.	Title	Page No.
Table (1):	Classification schemes for β-lactamases	6
Table (2):	Substrate and inhibition profiles of the carbapenemases	8
Table (3):	Interpretation of combination disk method	35
Table (4):	Antagonist of QS against selected bacteria and pathogens.	58
Table (5):	Zone diameter Interpretative standards for *P. aeruginosa*	68
Table (6):	Primers sequences used in this study	75
Table (7):	Components of reaction mixture for each 25 ul reaction for *bla*$_{KPC}$ genes (*bla*$_{KPC-1}$ through *bla*$_{KPC-7}$)	78
Table (8):	Components of reaction mixture for each 25 ul reaction for *bla*$_{IMP}$, *bla*$_{VIM}$ and *bla*$_{OXA-48}$	79
Table (9):	Patient demographic data of 100 CRPA isolates	88
Table (10):	Antimicrobial susceptibility pattern of 100 CRPA by disc diffusion susceptibility testing	89
Table (11):	Results of PCR detection of the four carbapenemase encoding genes on 100 CRPA isolates	93
Table (12):	Results of PCR genes for detection of carbapenemase encoding genes according to no. of genes detected in each isolates**Error! Bookmark not defined.**	4
Table (13):	Results of MHT compared to PCR	95
Table (14):	Results of PAE-MHT compared to PCR	96
Table (15):	Diagnostic performance of different phenotypic methods	96

List of Figures

Fig. No	Title	Page No.
Figure (1):	The modified Hodge test (MHT)	20
Figure (2):	Results of the modified Hodge test for a representative VIM-producing *P. aeruginosa* isolate with different indicator strains.	21
Figure (3):	Performances of DDSTs using disks of IPM or CAZ and disks of EDTA (1,900 µg) or 2-MPA	24
Figure (4):	Performances of DDSTs with different β-lactams and chelating agents	25
Figure (5):	MBL IPM/IPM-EDTA *E*-test for *P.aeuroginosa*	31
Figure (6):	Protocol for carbapenemase screening among *P. aeruginosa* isolates.	32
Figure (7):	Combination disk method: Representative results of the four combined disk tests using disks of meropenem (MEM) alone and with EDTA, 3 -aminophenylboronic acid, or EDTA plus 3-aminophenylboronic acid	34
Figure (8):	The Rapid Carb screen test with the use of different tubes and increasing inocula	37
Figure (9):	Examples of results for non-carbapenemase-producing and carbapenemase-producing *Pseudomonas* spp. from the Carba NP test	38
Figure (10):	Carba NP test II interpretation scheme	39
Figure (11):	Blue- Carba test	40
Figure (12):	*P. aeruginosa* on ChromID Carba.	70
Figure (13):	(A) MHT; isolates 1, 2, 4 show positive result, isolate 3 shows negative result. (B) PAE-MHT; isolates 1, 2, 3, 4 show positive result	73
Figure (14):	(A) MHT; isolates 1, 2 show indeterrminate result. isolate 3 shows negative result and isolate 4 shows positive result. (B) PAE-MHT; isolates 1, 2 show indeterrminate result. isolate 3 shows negative result and isolate 4 shows positive result	74

List of Figures (cont.)

Fig. No.	Title	Page No.
Figure (15):	Gel electrophoresis of PCR products	80
Figure (16):	Gel electrophoresis of multiolex PCR products	81
Figure (17):	Gel electrophoresis of KPC PCR products	82
Figure (18):	The antimicrobial susceptibility pattern of 100 CRPA by disc diffusion susceptibility testing	90
Figure (19):	The results of MHT, PAE-MHT and ChromID Carba on 100 CRPA isolates.	92
Figure (20):	The results of PCR for detection of four carbapenemases encoding genes on 100 CRPA isolates.	93

Introduction

Pseudomonas aeruginosa (P. aeruginosa) is an opportunistic pathogen causing severe invasive disease in critically ill and immunocompromised patients. Because of its ubiquitous nature, ability to survive in moist environments, and innate resistance to many antibiotics and antiseptics, it constitutes a common pathogen in hospitals ***(Cornaglia et al., 2000)***. Its treatment is a therapeutic challenge because of its intrinsic resistance to narrow spectrum β-lactams due to the constitutive expression of β-lactamases, efflux pumps and the low permeability of the outer membrane ***(Mesaros et al., 2007)***, in addition to the acquired production of the extended-spectrum β-lactamases (ESBLs) of the PER, VEB, and GES types ***(Nordmann and Naas, 2010)***. Consequently, the carbapenem-containing treatments were nearly the remaining option for its infections.

Carbapenem resistance in *P. aeruginosa* has been increased. It can result from different mechanisms, such as decreased bacterial outer membrane permeability, overexpression of AmpCs or expression of true carbapenemases ***(Dortet et al., 2014)***.

Carbapenemases production in *P. aeruginosa* belong to the Ambler class A (KPC- and GES-type β-lactamases) and most commonly the Ambler class B (metallo-β-lactamases) (MBLs) of the VIM, IMP, SPM, GIM, AIM, DIM, FIM, and NDM types ***(Dortet et al., 2014)***, also class D, the oxacillinases (e.g. OXA -48, -58) have been identified in *P. aeurogenosa* ***(Mataseje et al., 2012)***.

Screening of carbapenemase production among carbapenem resistant *P. aeruginosa* isolates is important since many carbapenemase genes are plasmid carried and easily transferable. So, Detection of those strains is of major importance for the determination of appropriate therapeutic schemes and the implementation of infection control measures ***(Pasteran et al., 2011a)***.

Several inhibitor-based tests have been developed for the detection of carbapenemases in *P. aeruginosa*. However, misdetection of newly emerging isolates with a combination of carbapenemases could occur with these methods ***(Pasteran et al., 2011a)***.

The modified Hodge test (MHT) has been widely used for carbapenemase screening by routine laboratories. Because of its simplicity, the Clinical and Laboratory Standards Institute ***(CLSI, 2011)*** has recommended its use in *Enterobacteriaceae* with elevated carbapenem MICs or reduced disk diffusion inhibition zones for detection of carbapenemases production. However, this recommendation does not include *P. aeruginosa*. Only two reports addressed the usefulness of the MHT using *P. aeruginosa* isolates of known genotype as the gold standard. Using the methodological standardization for *Enterobacteriaceae,* it was reported that the background lawn formation by *E. coli* ATCC 25922 was inhibited by a large proportion of the tested strains, defined as an equivocal or indeterminate result ***(Pasteran et al., 2011a)***. It is clear then, that the traditional MHT needs to be refined for use in *P. aeruginosa*, with the optimum test conditions defined to ensure a higher performance.

So the MHT was optimized for a more accurate and reliable detection of carbapenemase production in *P.*

aeruginosa by using a novel indicator strain, *K. pneumoniae* ATCC 700603, and this test was named the *P. aeruginosa* MHT (PAE-MHT) ***(Pasteran et al., 2011a)***.

Chromogenic media containing a carbapenem (chromID Carba, chromID KPC, chromID ESBL) are convenient tools for the screening and rapid detection of carbapenemase producing Gram-negative bacilli (CPGNB). Among the different chromogenic media designed to detect CPGNB, chromID Carba demonstrated the highest sensitivity and specificity ***(Simner et al., 2015).***

Different genotpic methods have been applied for detection of carbapemenases encoding genes in clinical isolates of *P. aeuroginosa* ***(Simner et al., 2015).***

Aim of the Work

The aim of the work is to:

1. Evaluate the ability of phenotypic methods (ChromID Carba agar chromogenic medium, Modified Hodge test (MHT) and *P. aeruginosa* -Modified Hodge test (PAE-MHT)) for detection of carbapenemase producing strains of *P. aeruginosa.*
2. Determine some carbapenamase gene classes associated with different *P. aeruginosa* carbapenam resistant isolates using conventional PCR (bla_{KPC}) and conventional multiplex PCR (bla_{VIM}, bla_{IMP}, $bla_{OXA\text{-}48}$).

Carbapenemases

Excessive use of carbapenems (because of ESBLs spread) has led to the emergence of carbapenemases ***(Bălăşoiu et al., 2014).***

Spread of carbapenemase-producing *P. aeruginosa* strains is a critical issue since those strains are resistant to almost all β-lactams. In addition, the early detection of carbapenemases is important because carbapenemase genes are usually located on transferable genetic determinants such as plasmids, leading to their rapid dissemination ***(Dortet et al., 2014).*** So, Detection of those strains is of major importance for the determination of appropriate therapeutic schemes and the implementation of infection control measures ***(Pasteran et al., 2011b).***

Carbapenemases can be classified on the basis of function or structure. The most commonly used classification is Ambler which based on molecular structure. In this classification, carbapenemases belong to classes A, B, or D. Class A and D are serine carbapenemases, meaning that they have a serine at their active sites, like ESBLs. In contrast, the class B enzymes are known as metallo-β-lactamases, because they require zinc as a cofactor. Ambler classified beta-lactamases according to the amino acid sequences. While according to Bush-Jacoby functional classification carbapenemases fall under groups 2f, 2df and 3, this classification depend on biochemical analysis of enzyme, determination of isoelectric point, determination of substrate hydrolysis, enzyme kinetics and inhibition profiles ***(Sridhar, 2012)*** (Table1) ***(Bassetti et al., 2011).***