Production of Metallo-β-Lactamases among Carbapenems Resistant Aerobic Non-Fermentative Gram-Negative Bacilli from Hospitalized Patients at Kasr El-Aini Hospital

Thesis

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Presented by

Alaa Mohamed Reda Mohamed Awad
M.B;B.Ch., M.Sc. (2004).

Supervised By

Prof. Dr Abdel Fattah Mohamed Attia
Professor of Medical Microbiology and Immunology

Prof. Dr Hicham Ezzat Mohamed Mokbel Ezzat
Professor of Medical Microbiology and Immunology

Dr Shourok Khamis Mohamed
Assistant Professor of Medical Microbiology and Immunology

Dr Iman Ezzat Wali
Assistant Professor of Medical Microbiology and Immunology

Faculty of Medicine
Cairo University
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Abstract

MBLs constitute the most clinically important group of carbapenemases, as they are capable of hydrolyzing all β-lactams, including carbapenems except the monobactam aztreonam. Acquired MBLs are emerging resistance determinants in *P. aeruginosa* and other non-fermentative gram-negative bacilli. They disseminate rapidly through bacterial population causing a threat to treatment with β-lactam antibiotics and carbapenems.

The aim of this study was to determine the prevalence of carbapenems resistance among non-fermentative gram-negative bacilli and the prevalence of MBLs-producing isolates among carbapenems-resistant ones.

Screening for carbapenems-resistant isolates among 73 non-fermentative gram-negative bacilli was done by disk diffusion method. Detection of MBLs among resistant ones was done by both phenotypic methods as IPM-EDTA combined disk method and MBL E-test and genotypic method as PCR.

Results showed that the prevalence of IPM resistance among non-fermentative gram-negative bacilli was 57% with the greatest prevalence rate for *P. aeruginosa* 88%. The prevalence of MBL-producing isolates among IPM-resistant ones was 26% by both phenotypic and genotypic methods, while it was 74% by phenotypic methods which is a very high rate.

Key words:

Non-fermentative gram-negative bacilli

P. aeruginosa

MBLs

IPM-EDTA combined disk

MBL E-test

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List of Abbreviations

AHLs	Acylhomoserine lactones
ARI-1	*Acinetobacter* resistant to imipenem
ABC	ATP-binding cassette family
BCII	Beta-lactamases from *Bacillus cereus*
β	Beta
CAZ	Ceftazidime
CcrA (CfiA)	Enzyme from *Bacteroides Fragilis*
CLSI	Clinical and Laboratory Standard Institute
CphA	Enzymes from *Aeromonas hydrophilia*
CTX-M-ases	Cefotaxime hydrolyzing enzymes
DDST	Double disk synergy test
DSF	Diffusible signalling factor
EDTA	Ethylene diamine tetra acetic acid
ESBL	Extended-spectrum β-lactamase
FEZ-1	Enzyme from *Legionella gormanii*
GES	Guiana extended spectrum
GIM-1	German imipenemase
GOB	Enzyme from *Chryseobacterium meningosepticum*
IMI	Imipenem-hydrolyzing ß-lactamase
IMP	Active on imipenem
IND-1	Enzyme from *Chryseobacterium indologenes*
IP	Imipenem

IPI	Imipenem EDTA
IPM	Imipenem
KPC	*Klebsiella pneumoniae c*arbapenemase
L1	Enzyme from *Stenotrophomonas maltophilia*
MBLs	Metallo-ß-lactamases
MPA	Mercaptopropionic acid
NCCLS	National Committee for Clinical Laboratory Standards
NMC	Not metallo **e**nzyme carbapenemase
OXA	Oxacillin hydrolyzing enzymes
PFGE	Pulsed-field gel electrophoresis
QS	Quorum sensing
RND	Resistance-nodulation-division family
Sfh-I	ß-lactamases from *Serratia fonticola*
SHV	Sulfhydryl variable
SIM-1	Seoul imipenemase
SMA	Sodium mercaptoacetic acid
SME	*Serratia marcescens* enzyme
SPM	Sao Paulo metallo-ß-lactamase
TBE	Tris-borate EDTA
TEM	Temoniera (patient's name of the 1st isolate)
THIN-B	Enzyme from Janthinobacterium lividum
VIM	Verona integron-encoded metallo-ß-lactamase

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Introduction and Aim of Work

Non-fermentative gram-negative bacilli, particularly *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are niche pathogens that cause opportunistic infections in patients who are critically ill or immunocompromised, particularly patients in intensive care units ***(Vidal et al., 2003)***. Systemic infections due to non-fermentative gram negative bacilli have become increasingly more frequent in recent years and are usually difficult to treat because they represent the problem of multidrug resistance. Non susceptibility of gram negative non-fermenters is typically due to both intrinsic resistance and rapidly acquired resistance ***(McGowan, 2006)***.

Several enzymes that can inactivate and modify many antibiotics are produced by non-fermenters, as large number of β-lactamases. β-Lactamases are a heterogeneous group of proteins with structural similarities. They are composed of α-helices and a β pleated sheet ***(Knox, 1995)***. Two schemes are currently used to classify β-lactamases: the Ambler classification scheme and the Bush-Jacoby-Medeiros classification system ***(Bush, 2001)***. The Ambler scheme separates β-lactamases into four distinct classes based on similarities in amino acid sequence. Classes A, C and D are serine β-lactamases, whereas class B enzymes are metallo-β-lactamases (MBLs). The Bush-Jacoby-Medeiros scheme classifies β-lactamases according to functional similarities (substrate and inhibitor profiles). There are four categories: group 1 cephalosporinases, group 2 penicillinases, group 3 MBLs, and group 4 as well as multiple subgroups (2a, 2c, 3a, etc.) ***(Murray et al., 2003)***.

Metallo-β-lactamases have recently become more prominent among the β-lactam-hydrolyzing enzymes. They require zinc or another heavy metal for catalysis and their activities are inhibited by chelating agents. They are resistant to inactivation by clavulanate, sulbactam, and tazobactam ***(Rasmussen and Bush, 1997)***. These clavulanic-acid resistant enzymes have a large spectrum of hydrolysis including penicillins, cephalosporins (third and fourth generations), carbapenems but not monobactams ***(Nordmann and Poirel, 2002)***. They can be grouped into three different subclasses; B1, B2, and B3 ***(Galleni et al., 2001)***.

Subclass B1 contains the MBL (BcII) from *Bacillus cereus*, the CcrA β-lactamase from *Bacteroides fragilis* and the BlaB β-lactamase from *Chryseobacterium meningosepticum*. Subclass B1 also contains the IMP β-lactamase found in some clinical isolates of *Pseudomonas aeruginosa*, *Acinetobacter baumannii, Serratia marcescens, and Klebsiella pneumoniae*, and the VIM β-lactamase found in some strains of *Pseudomonas aeruginosa* ***(Laraki et al., 1999*** and ***Poirel et al., 2000[b])***.

Subclass B2 includes β-lactamases produced by various species of *Aeromonas* (CphA, ImiS, and CphA2) and the Sfh-1-β-lactamase from *Serratia fonticola* ***(Saavedra et al., 2003)***.

Finally, subclass B3 includes the L1 β-lactamase from *Stenotrophomonas maltophilia*, the GOB β-lactamase from *Chryseobacterium meningosepticum*, the FEZ-1 β-lactamase from *Legionella gormanii* and the THIN-B β-lactamase produced by *Janthinobacterium lividum* ***(Galleni et al., 2001)***.

MBLs, like all β-lactamases can be divided into those that are normally chromosomally mediated and those that are encoded by transferable genes ***(Walsh et al., 2005)***. The early studies on chromosomally mediated MBLs mainly centered around (BC II) and (L1). However, primarily due to genomic sequencing, increasingly more chromosomally mediated genes are being discovered but are often found in obscure nonclinical bacteria ***(Saavedra et al., 2003)***. Moreover, during the past 3 to 4 years, many new transferable types of MBLs have been studied and appear to have rapidly spread ***(Gales et al., 2003)***. The spread of MBLs in gram negative rods has been described in several countries and is becoming an emerging threat ***(Tsakris et al., 2000)***.

Carbapenems are often used as antibiotics of the last resort for the treatment of infections caused by gram-negative bacteria resistant to other β-lactam agents. This is due to their broad spectra of activity and their stability to hydrolysis by most β-lactamases including extended-spectrum β-lactamases ***(Yan et al., 2001)***. However, *Pseudomonas aeruginosa* often develops resistance to carbapenems as a result of reduced levels of drug accumulation or increased levels of expression of pump efflux ***(Matsumura et al., 1999)***. Their resistance to carbapenems may occasionally be due to production of MBLs, which can either be chromosomally encoded or plasmid mediated ***(Poirel et al., 2000)***[a].