Detection of New Delhi Metallo-beta Lactamase Gene among Carbapenem Resistant Gram Negative Bacilli

Thesis

Submitted for Partial Fulfillment of the M.D Degree In Clinical Pathology

Presented by

Nouran Hassan Mohamed Fawzy Madkour

MB BCh, M Sc Clinical Pathology

Faculty of Medicine, Ain Shams University

Supervised by

Professor/ Amira Mohamed Mokhtar

Professor of Clinical Pathology

Faculty of Medicine - Ain shams University

Professor/ Malaka Zakaria Amer

Professor of Clinical Pathology

Faculty of Medicine - Ain shams University

Professor/ Hala Mahmoud Hafez

Professor of Clinical Pathology

Faculty of Medicine - Ain shams University

Doctor/ Marwa Abd El Rasoul El-Ashry

Lecturer of Clinical Pathology

Faculty of Medicine - Ain shams University

Faculty of Medicine

Ain Shams University

2018

سورة البقرة الآية: ٣٢

Acknowledgment

First and foremost, I feel always indebted to **ALLAH**, *the Most Kind and Most Merciful.*

I'd like to express my respectful thanks and profound gratitude to **Professor/ Amira Mohamed Mokhtar,** *Professor of Clinical and Chemical Pathology - Faculty of Medicine- Ain Shams University for her keen guidance, kind supervision, valuable advice and continuous encouragement, which made possible the completion of this work.*

I am also delighted to express my deepest gratitude and thanks to **Professor/ Malaka Zakaria Amer,** *Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

Many thanks and sincere appreciation **Professor/ Hala Mahmoud Hafez,** *Professor of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her great help, active participation and guidance.*

I am deeply thanks to **Doctor/ Marwa Abd El Rasoul El-Ashry,** *Lecturer of Clinical and Chemical Pathology, Faculty of Medicine, Ain Shams University, for her kindness, supervision and cooperation in this work.*

I would like to dedicate this work to **my Family** *who have always been my support and my backbone and without whom I wouldn't have been where I am today. Thank you for always being there for me and for loving me unconditionally.*

Nouran Madkour

List of Contents

Title	Page No.

List of Abbreviations i

List of Figures iv

List of Tables vi

Introduction 1

Aim of the Work 4

Review of Literature

📖 β-Lactamases 5

📖 Laboratory Detection of Metallo- β -Lactamases 15

📖 Treatment 31

📖 Prevention and Control of Antimicrobial Resistant Bacteria 39

Materials and Methods 46

Results 60

Discussion 75

Summary 85

Conclusion and Recommendations 89

References 93

Arabic Summary

List of Abbreviations

Abb.	Full term
AMA	*Aspergillomarasmine A*
AmpC	*Ambler Class C*
BCII	*Bacillus cereus metallo beta lactamase*
bla	*Beta -lactamase*
Bst	*Bacillus Sterothermophillus*
CAI	*Community Acquired Infection*
CAZ	*Ceftazidime*
CDC	*Center for Disease Control and Prevention*
CLSI	*Clinical Laboratory Standard Institute*
CMS	*Colistimethate*
CPE	*Carbapenemase Producing Enterobacreiaceae*
cphA	*Aeromonas Hydrophilia Spp. metallo* β *Lactamase*
CRE	*Carbapenem-resistant Enterobacteriaceae*
Ct	*Cycle threshold*
CTX-Ms	*Cefotaximase*
E.aerogenes	*Enterobacter aerogenes*
E.cloacae	*Enterobacter cloacae*
E.coli	*Escherichia coli*
EDTA	*Ethylene diamine tetra-acetic acid*
ESBL	*Extended spectrum* β*-lactamases*
ESCMID	*European Society of Clinical Microbiology and Infectious Diseases*
E-Test	*Epsilometer Test*
ETP	*Ertapenem*
EUCAST	*European Committee on Antimicrobial Susceptibility Testing*
FEZ-1	*Fluoribacter Gormanii Enzyme*
GES	*Guiana extended-spectrum*

List of Abbreviations (Cont...)

Abb.	Full term
GIM	*German imipenemase*
Gob-1	*Elizabethkingia meningoseptica enzyme*
HAI	*Hospital acquired infections*
HCWs	*Health care workers*
HGT	*Horizontal gene transfer*
HS	*.Highly significant*
ICUs	*Intensive care units*
IEF	*Isoelectric Focusing*
IMI	*Imipenem-hydrolysing B-lactamase*
IMP	*Active on Imipenem*
IMP-EDTA	*Imipenem-EDTA*
KPC	*Klebsiella pneumonia carbapenemase*
L1	*Stenotrophomonas maltophilia*
LAMP	*Loop- mediated Isothermal Amplification*
M	*Molar*
MALDI-TOF.MS	*Matrix assisted laser - desorption ionization-time of flight mass spectrometry*
MBLs	*Metallo-βlactamases*
MDR	*Multi drug resistant*
MEM	*Meropenem*
MHT	*Modified Hodge Test*
MIC	*Minimum inhibitory concentration*
MPA	*Mercaptopropionic acid*
NDM	*New Delhi metallo-βlactamase*
NGS	*Next Generation Sequencing*
NMC	*Not metalloenzyme carbapenemase*
NS	*Non-significant*
OXA	*Oxacillinases*

List of Abbreviations (Cont...)

Abb.	Full term
PC	*Personal Computer*
PCR	*Polymerase chain reaction*
RT-PCR	*Real time polymerase chain reaction*
Sfh-1	*Serratia fonticola enzyme*
SHV	*Sulfhydryl variable*
Sig	*Significant*
SME	*Serratia marcescens enzyme*
SPM	*Sao Paulo metallo βlactamase*
TEM	*Temoneira (name after the patient providing the first sample)*
TSB	*Trypticase soy broth*
UK	*United Kingdom*
VIM	*Verona integrin-encoded metallo-B-lactamase*
WGS	*Whole Genome Sequencing*

List of Figures

Fig. No.	Title	Page No.
Figure (1):	Modified Hodge Test	20
Figure (2):	E-test showing carbapenemase	22
Figure (3):	Disk approximation test	23
Figure (4):	Positive imipenem-EDTA combined disc test	24
Figure (5):	Representative results of the Carba NP test	25
Figure (6):	Representative results of the Carba NP test II interpretation scheme	26
Figure (7):	Rotor –Gene Q.	51
Figure (8):	An isolate of MBL producing Gram negative bacillus showing positive IMP-EDTA disk test.	53
Figure (9):	A positive isolate for *bla*$_{NDM-1}$.	57
Figure (10):	A negative isolate for *bla*$_{NDM-1.}$	57
Figure (11):	Real-time PCR melting curves of the amplicon generated by primers targeting *bla*$_{NDM-1}$	58
Figure (12):	The studied carbapenem-resistant isolates.	60
Figure (13):	Types of clinical samples from which the carbapenem-resistant isolates were isolated.	61
Figure (14):	Frequency isolation of carbapenem-resistant isolates among different hospital departments.	62

List of Figures (Cont...)

Fig. No.	Title	Page No.
Figure (15):	The most commonly administered antibiotics.	64
Figure (16):	Frequency Distribution of Inserted Device among Patients.	65
Figure (17):	Frequency of MBL producing isolates among the carbapenem-resistant isolates.	66
Figure (18):	Positive isolates for *bla*$_{NDM-1}$.	74

List of Tables

Table No.	Title	Page No.
Table (1):	Substrate and inhibition profiles of the carbapenemases	7
Table (2):	EUCAST 2015 and CLSI 2016 guidelines for zone diameter by disc diffusion method	16
Table (3):	EUCAST 2015 and CLSI 2016 guidelines for MIC by broth microdilution method	17
Table (4):	Components of reaction master mix for each 25 ul reaction	56
Table (5):	Cycler program	56
Table (6):	Source of infection and history of previous hospitalization	63
Table (7):	Association between the type of isolate and MBL production	67
Table (8):	Comparison between non-fermenters and Enterobacteriaecae regarding the MBL production	67
Table (9):	Association between the type of infection and isolation of MBL producing strains	68
Table (10):	Comparison between the different hospital departments regarding the isolation of MBL producing strains among carbapenem resistant organisms	69
Table (11):	Association between MBL production among hospital associated and community acquired infections.	70
Table (12):	Association between the presence of medical device and isolation of MBL producing strains	71

List of Tables (Cont...)

Table No.	Title	Page No.

Table (13): Association between MBL production and previous hospitalization 72

Table (14): Association between antibiotic intake and the isolation of MBL producing strains 73

ABSTRACT

Out of the 41 MBL positive patients, 63.4% had no medical device inserted whereas 36.6% had at least one medical device inserted. No statistically significant association was observed between the presence of a medical device and the isolation of MBL producing strains (*P>0.05*).

No statistically significant association was found between previous hospital admission and isolation of MBL producing strains (*P > 0.05*). Most of the MBL producing strains were isolated from patients with no history of previous hospital admission (75.6%) whereas only 10 MBLs producing strains (24.4%) were isolated from patients with history of prior hospitalization.

Finally, although most of the MBL producing isolates were recovered from patients taking antibiotics (85.4%), no statistically significant association was found between antibiotic intake and the isolation of MBL producing strains (*P > 0.05*).

Keywords: Carbapenem-resistant Enterobacteriaceae - Aeromonas Hydrophilia Spp. metallo β Lactamase - Carbapenemase Producing Enterobacreiaceae

INTRODUCTION

Rapid expanding multidrug resistant bacteria are a major public health problem causing both nosocomial and community-acquired infections. One of the most important emerging traits is resistance to extended-spectrum B-lactams in Gram-negative microorganisms ***(Nordmann et al., 2012a).*** The widespread use of carbapenems, the only agents reliably active against these bacteria, led to the emergence of a new resistance mechanism ***(Zarfel et al., 2011).***

Carbapenems - hydrolysing β - lactamases, i.e., Carbapenemases, in bacterial clinical isolates are an increasing concern because they often also confer resistance to most other β-lactam antimicrobial agents. Among *Enterobacteriacae*, carbapenemases are found mainly in the Ambler class A (serine β-lactamases) or class B (metallo- β-lactamase) groups ***(Mulvey et al., 2011).*** The different mechanisms of these families account for their different behavior with metal chelators e.g., with ethylene diamine tetraacetic acid (EDTA), which do not affect the activity of serine-β-lactamases, but do inhibit metallo-β- lactamases **(*Cornaglia et al., 2011).***

The New Delhi metallo-β-lactamase (NDM) is a transferable molecular class B β-lactamase that was first recovered from *Klebsiella pneumoniae* and *Escherichia coli*, isolated in Sweden in 2008, from an Indian patient transferred

one day previously from a New Delhi hospital ***(Yong et al., 2009).*** The enzyme was found to be present largely in Enterobacteriaceae, but also in non-fermenters and *Vibrionaceae*. Since its discovery the NDM has become a source of serious concern. It has been identified in the UK, India and Pakistan ***(Nordmann et al., 2011)***, the Sultanate of Oman, Morocco, Algeria, Iraq, Egypt, Taiwan, America, France, Kenya, Italy, Japan, the Netherlands, Norway, Morocco, Lebanon and Emirates ***(Fallah et al., 2011; Kaase et al., 2011; Poirel et al., 2011a; Liang et al., 2011; Sidjabat et al., 2011).***

Most NDM-positive bacteria are broadly resistant to all beta-lactam antibiotics and very often carry on the same transposon the genes responsible for resistance to trimethoprim-sulfamethoxazole, aminoglycosides and fluoroquinolones which make the treatment of patients infected with such organisms very difficult**.** Only aztreonam, tigecycline, colistin and fosfomycin can be effective but these also have limitations ***(Anthony et al., 2008).*** Thus, reliable detection and active surveillance are crucial to prevent the dissemination of such resistance ***(Perez et al., 2007).***

A series of non-molecular-based tests [e.g., modified Hodge test (MHT) and inhibition studies by EDTA] have been proposed for detection of carbapenemase activity. However

none of the currently available tests has 100% specificity or 100% sensitivity ***(Nordmann et al., 2012b).*** Moreover, a variety of culture techniques have been proposed for screening of carbapenem resistant isolates including commercially available chromogenic agar media ***(Virioni et al., 2012).***

Molecular techniques remain the reference standard for the identification and differentiation of carbapenemases. Most of them are based on polymerase chain reaction (PCR), and may be followed by sequencing for precise identification of carbapenemase variants (e.g. *VIM*-type, *KPC*-type, *NDM*-type, and *OXA-48*-type). The PCR technique performed on colonies can give results within 4–6 hours or less when real-time PCR technology is used, with excellent sensitivity and specificity ***(Chen et al., 2011).***