Rapid Detection of Carbapenemase Producing Bacterial Isolates

Thesis

Submitted for Partial Fulfillment of the M.D Degree

In Clinical Pathology

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2016

سورة البقرة الآية: ٣٢

Acknowledgments

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 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/ Eman Mohamed Kamel,*** *Professor of Clinical Pathology Faculty of Medicine - Ain shams University, for her kind care, continuous supervision, valuable instructions, constant help and great assistance throughout this work.*

I am deeply thankful to ***Doctor/ Sherin Ahmed El-Masry****, Assistant Professor of Clinical Pathology, Faculty of Medicine - Ain shams University, for her great help, active participation and guidance.*

I wish to introduce my deep respect and thanks to ***Doctor/ Dalia Hosni Abd El-Hamid****, Lecturer of Clinical Pathology, Faculty of Medicine - Ain shams University, for her kindness, supervision and cooperation in this work.*

I would like to express my deep thanks to ***Doctor/ Hoda Ezz-El Arab****, Consultant of Clinical Pathology, Faculty of Medicine - Ain shams University, for her kind support till this work was completed.*

Last but not least my sincere thanks and appreciation to all my family for their support till this work was completed.

Maha Ahmed Anwar Hafez

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List of Abbreviations

Abb.	Full term
A. baumannii	Acinetobacter baumannii
AIM	Australian Imipenemase
APBA	3-AminoPhenyl Boronic Acid
aPI	Acute Pelvic Infections
BA	Boronic Acid
CAP	Community Acquired Pneumonia
CHDLs	Carbapenem Hydrolyzing class D β-Lactamases
cIAI	Complicated Intra-Abdominal Infections
CLSI	Clinical and Laboratory Standards Institute
CRE	Carbapenem Resistant Enterobacteriaceae
cSSSI	Complicated Skin and Skin Structure Infections
cUTI	Complicated Urinary Tract Infection
DIM	Dutch Imipenemase
E. asburiae	Enterobacter asburiae
E. coli	Escherichia coli
E. faecium	Enterococcus faecium
E.cloacae	Enterobacter cloacae
E.faecalis	Enterococcus faecalis
EDTA	Ethylene Diamine Tetra-acetic Acid
ESBL	Extended Spectrum β-Lactamase
EUCAST	European Committee on Antimicrobial Susceptibility Testing
FDA	Food and Drug Administration

List of Abbreviations (Cont...)

Abb.	Full term
GES	Guiana Extended Spectrum
GIM	German Imipenemase
GNB	Gram Negative Bacilli
H.influenzae	Haemophilus influenzae
HAP	Hospital Acquired Pneumonia
HMM	High Molecular Mass
HS	Highly Significant
IEF	Isoelectric Focusing
IMI	Imipenem hydrolyzing β-lactamase
IMP	Active on Imipenem
K.pneumoniae	Klebsiella pneumoniae
KHM	Kyorin Health Science Metallo-ß-lactamase
KPC	Klebsiella Pneumoniae Carbapenemase
LAMP	Loop Mediated Isothermal Amplification
LMM	Low Molecular Mass
MALDI-TOF	MS Matrix Assisted Laser Desorption Ionization Time of Flight Mass Spectrometry
MBLs	Metallo-ß-Lactamases
MHT	Modified Hodge Test
MICs	Minimal Inhibitory Concentrations
MRSA	Methicillin Resistant Staphylococcus Aureus
MSSA	Methicillin Sensitive Staphylococcus Aureus
NDM	New Delhi Metallo-ß-lactamase

List of Abbreviations (Cont...)

Abb.	Full term
NGS	Next Generation Sequencing
NMC	Non Metalloenzyme Carbapenemase
NS	Non Significant
NPV	Negative Predective Value
OMPs	Outer Membrane Proteins
OXA	Oxacillinases
P. aeruginosa	Pseudomonas aeruginosa
PAE-MHT	Pseudomonas Aeruginosa Modified Hodge Test
PBPs	Penicillin Binding Proteins
PCR	Polymerase Chain Reaction
PPV	Positive Predective Value
RTI	Respiratory Tract Infections
S. maltophilia	Stenotrophomonas maltophilia
S.marcescens	Serratia marcescens
S.pneumoniae	Streptococcus pneumoniae
S.pyogenes	Streptococcus pyogenes
SIM	Seoul Imipenemase
SME	Serratia Marcescens Enzyme
SPM	Sao Paulo Metallo-ß-lactamase
S	Significant
SSI	Surgical Site Infection
VAP	Ventilator Associated Pneumonia
VIM	Verona Integron encoded Metallo-ß-lactamase
WGS	Whole Genome Sequencing

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Abstract

Carba NP and CarbAcineto NP tests are highly sensitive and specific, cheap, rapid tests for the detection of carbapenem resistance due to carbapenemase production. So implementation of infection control measures can be achieved to prevent their further dissemination.

CHROMagar KPC is an easy screening method for the detection of carbapenem resistance, however it lacks specificity in detection of carbapenemase production.

Although PCR is a rapid and highly sensitive method in detection of Carbapenemase production, it is very expensive and needs trained personnel.

Key words: High Molecular Mass- Hospital Acquired Pneumonia- Haemophilus influenzae- Klebsiella pneumoniae- Kyorin Health Science Metallo-β-lactamase- Low Molecular Mass- Modified Hodge Test- Metallo-β-Lactamases.

INTRODUCTION

Carbapenems are commonly considered as last-resort antibiotics in the treatment of severe infections caused by multidrug-resistant gram negative bacteria. Over the past decade, the emergence of carbapenem-resistant isolates is very unnerving, since it contributes to the reduction of the current therapeutic pallet, therefore leaving few or, in some cases, no optimal therapeutic options ***(Voulgari et al., 2013).***

Increased carbapenem MICs in gram-negative bacteria can be a result of two different mechanisms of resistance: hyperproduction of class C β-lactamases or extended- spectrum β-lactamases (ESBLs) in combination with porin alteration; and/or carbapenemase production ***(Monterio et al., 2012).*** Although several mechanisms of carbapenem resistance have been reported, most of the mechanisms are related to the spread of carbapenemases belonging to Ambler class A (KPCs), class B (VIMs, IMPs, and NDM-1), and class D (OXA-48) β-lactamases ***(Nass et al., 2011).***

Klebsiella pneumonia carbapenemases (KPCs) have been found in bacteria other than Klebsiella pneumoniae, including Klebsiella oxytoca, Proteus mirabilis, Acinetobacter spp, Pseudomonas aeruginosa, Citrobacter freundii, Serratia marcescens and Escherichia coli. Bacterial isolates producing KPCs are able to hydrolyze a broad spectrum of β-lactams including the penicillins, cephalosporins, carbapenems and

monobactam. They have the potential to spread rapidly in hospital environments to cause nosocomial infections with high mortality rates ***(Wang et al., 2012).***

Rapid and accurate detection of carbapenemase-producing strains is pivotal for adequate antibiotic therapy and infection control, especially in an outbreak setting ***(Stuart et al., 2012).***

The detection of carbapenemases primarily on the basis of phenotypic testing may sometimes be difficult, since the MICs of carbapenem drugs may remain in the susceptibility range. Detection and identification of these carbapenem-resistant isolates may be improved significantly by the use of automated susceptibility testing systems ***(Nordmann et al., 2011).***

A series of non-molecular-based tests (e.g. modified Hodge test (MHT) and inhibition studies by EDTA and dipicolinic acid) have been proposed for detection of carbapenemase activity. None of the currently available tests has 100% specificity or 100% sensitivity ***(Nordmann et al., 2012b).***

A variety of culture techniques have been proposed for screening of carbapenem resistant isolates including both in-house-prepared selective media in broth or solid agar form ***(Calfee and Jenkins, 2008)*** and commercially available agar plates either chromogenic or non- chromogenic media ***(Virioni et al., 2012).***