Genotypic characterization of carbapenem resistant Gram negative bacteria

Thesis submitted for Partial Fulfillment of Master Degree in Microbiology

By

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B.Sc. Microbiology special course, 2008

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Approval Sheet

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Abbreviations

ABC complex	*A.baumannii complex*
ACB complex	*A. calcoaceticus* - *A. baumannii* complex
AMEs	Aminoglycoside-Modifying Enzymes
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
BAL	Broncoalveolar Lavage
BSI	Blood Stream Infection
CAZ	Ceftazidime
CCU	Coronary care unit
CDC	Centre for Disease Prevention and Control
CHDL	Carbapenem Hydrolyzing Class D β-Lactameases
CIP	Ciprofloxacin
CLSI	Clinical Laboratory Standards institute
CRAB	Carbapenem Resistance *Acinetobacter baumannii*
CRGNB	Carbapenem-Resistant Gram-Negative bacteria
CSF	Cereprospinal fluid
CTX	Cefotaxime
CVL	Central Venous Line
CXM	Cefuroxime
DHP-I	Dehydropeptidase I
EARS-Net	European Antimicrobial Resistance Surveillance Network
ECDC	European Centre for Disease Prevention and Control
EPIC	Epidemiologists and Preventionists in Infection Control organization
ESBL	Extended Spectrum β-lactamase
ETT	Endotracheal tube
FEMS	Federation of European Microbiological Societies
G+C content	Percentage of DNA consisting of guanine and cytosine bases
GES	Guiana extended spectrum

GN	Gentamicin
HAIs	Healthcare Acquired Infections
ICU	Intensive Care Unit
IHMA	International health management association
IMI	Imipenem hydrolyzing carbapenemase, (Class A)
IMP	Imipenem hydrolyzing carbapenemase, metallo-β-lactamase (Class B)
IPC	Infection Prevention and Control
IPM	Imipenem
IS	Insertion Sequence
IS*CR*s	Insertion Sequence Common Region
JAMA	Journal of American Medical Association
KPC	*Klebsiella pneumonia* carbapenemase
MBLs	Metallo-β-lactamases; Ambler class B
MDR	Multidrug Resistant
MDROs	Mutidrug Resistant Organisms
MEM	Meropenem
MICs	Minimum Inhibitory Concentrations
MLSA	Multilocus Sequences Analysis
MLST	Multilocus Sequence Typing
MOX	Moxifloxacin
MRGN	Multidrug-Resistant Gram-Negative bacteria
MRSA	Methicillin resistant *Staphylococcus aureus*
NDM	New-Delhi Metallo-β-lactamase
NICU	Neonate Intensive Care Unit
NMC-A	Non Metallo-carbapenemase
NNIS	National Nosocomial Infection Surveillance center
NNSN	National Healthcare Safety Network
OMPs	Outer Membrane Proteins
OXAs	Oxacillinases; Ambler Class D
PBPs	Penicillin Binding Proteins
PDR	Pan-Drug Resistant

PICU	Pediatric Intensive Care Unit
PPL	Priority Pathogens List
RTI	Respiratory Tract Infection
SAM	Ampicillin/sulbactam,
SME	*Serratia marcescens* carbapenemase
SSI	Surgical Site Infection
SXT	Trimethoprim/sulfamethoxazole
T_{ann}	Annealing temperature
TE	Tetracycline
TEST	Teigecycline Evaluation Surveillance Trial
TGC	Tigecycline
T_m	Melting temperature
TZP	Piperacillin/tazobactam
UTI	Urinary Tract Infection
VAP	Ventilator-Associated Pneumonia
VIM	Verona Integron-encoded Metallo-β-Lactamase
VRE	Vancomycin Resistant *Enterococcus*
XDR	Extensive Drug Resistant

Abstract

Multidrug-resistant Gram-negative bacteria (MRGN) are a serious threat and a challenge to the healthcare system. Carbapenems have been used as "antibiotics of last resort" for treatment of multidrug resistant (MDR) bacteria. Unfortunately, the emergence of carbapenem-resistant Gram-negative pathogens (CRGNB) has increased dramatically in the last decade threatening this class of lifesaving drugs. In this study, 232 Gram negative pathogens were collected and screened for carbapenem resistance. *Acinetobacter baumannii* was found to be the highest carbarpenem resistant pathogen. Forty carbapenem resistant *A.baumannii* isolates were screened for the four main OXA-type carbapenemase sub-groups by PCR. The co-existence of multiple OXA-type genes was reported for the first time in Egyptian isolates of *A.baumannii*. The co-existence of multiple OXA genes were found in all the tested CRAB isolates, 75% were OXA-23/OXA-24/OXA-51 carriers, 10% were OXA-23/OXA-51 carriers, 10% were OXA-24/OXA-51 carriers and 5% were OXA-23/OXA-24 carriers. This study provides an alarming epidemiological snapshot on the distribution of OXA-type carbapenemes in Egypt.

Introduction:

Infections with Multidrug-resistant Gram-negative bacteria (MRGN) are a serious threat and a challenge to the healthcare system. Carbapenems (imipenem and meropenem) have been used as “last-line agents” or “antibiotics of last resort” for treatment of MDR bacteria such as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and *Enterobacteriaceae* producing extended-spectrum β-lactamase (ESBL) in hospital settings due to their high potency, broad-spectrum activity and safety profile **(Rodloff, 2006)**. Unfortunately, the emergence of Carbapenem-resistant Gram-negative pathogens (CRGNB) has increased dramatically in the last decade threatening this class of lifesaving drugs. (**Akova *et al.*, 2012**).

Carbapenem-resistant Gram-negative bacteria (CRGNB) have heightened global awareness of the prioritization of Infection prevention and control interventions (IPC) to minimize these infections. The World Health Organization (WHO) developed a global priority pathogens list (global PPL) of antibiotic-resistant bacteria to help in prioritizing the research and development of new and effective antibiotic treatments. The pathogens were grouped according to the species and the type of resistance into three priority categories: critical, high and medium. The critical priority category included carbapenem-resistant *Acinetobacter baumannii*, (ranked as no.1), carbapenem-resistant *Pseudomonas aeruginosa and* carbapenem-resistant *and* third generation cephalosporin-resistant *Enterobacteriaceae* including: *Klebsiella pneumonia*, *Escherichia coli*, *Enterobacter* spp., *Serratia* spp., *Proteus* spp., and *Providencia* spp, *Morganella* spp.) **(WHO, 2017).**

The most common mechanism of carbapenem resistance is the production of carbapenem hydrolyzing β-lactamases, or carbapenemeases, which often confer resistance not only to carbapenems but to most β-lactam agents. Carbapenemases can be grouped into two major classes; class B (metallo β-lactamases; MBLs) and class D (oxacillinases; OXAs). These enzymes have the common property of hydrolyzing, imipenem or meropenem together with other penicillin or cephalosporin antibiotics. Despite MBLs exhibit more potent hydrolytic activity against carbapenems than OXAs (**Kim *et al.*, 2014**); OXAs are diverse, comprising over 220 enzymes and more frequently identified especially in *A. baumannii* isolates, whereas MBL production is more prevalent in other Gram negative pathogens and non-*baumannii* species **(Queenan and Bush, 2007; Lee *et al.*, 2009, b; Lee *et al.*, 2012; Higgins *et al.*, 2010, b)**

Carbapenem Resistance *Acinetobacter baumannii* (CRAB) outbreaks were increasingly reported in several parts of the world including developing countries **(Peleg *et al.*, 2008).** The success of *A.baumannii* as a nosocomial pathogen and its designation as a "red alert" pathogen is attributed to its extensive resistance antibiotic profile - resistant to almost all antibiotics available - and its predominance in health care settings due to the selective pressure of antibiotics and incredible ability to acquire resistance and colonize **(Maslow, 2005).**

Aim of work

In this study, we aimed to assess antimicrobial susceptability profile , evaluate the prevalence of carbapenem resistant among Gram negtive pathogens and study the genotypic variation of carbapenem resistant genes belonging to class D β-lactames (OXA type carbapenemases) in *A.baumannii* isolates from three Egyptian hospitals.