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٢٠١٤

GENOTYPIC DETECTION OF *OXA 23* IN CARBAPENEM RESISTANT *ACINETOBACTER BAUMANNII*

Thesis
Submitted for Partial Fulfillment of M.D Degree in Clinical and Chemical Pathology

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Karim Montasser

List of Abbreviations

ABC	:	ATP- Binding Cassette
AFLP	:	Amplified Fragment Length Polymorphism
Amp C	:	Ambler Class C Enzymes
API 20NE	:	Analytical Profile Index 20 Enterobacteria
AP- PCR	:	Arbitrarily Primed Polymerase Chain Reaction
ARDRA	:	Amplified 16S Ribosomal DNA (rDNA) Restriction Analysis
CA- BSI	:	Catheter Associated Blood Stream Infection
CDC	:	Centers for Disease Control and Prevention
CLSI	:	Clinical Laboratory Standard Institute
CMY	:	Cephalosporins Mediated by beta- Lactamases
CM	:	Cytoplasmic Membrane
CTX	:	Cefotaximase
CRGNB	:	Carbapenem- Resistant Gram Negative Bacilli
EDTA	:	Ethylene- Diamine- Tetra- Acetic Acid
ESAC	:	Extended Spectrum Amp C
ESBL	:	Extendend - Spectrum β Lactamases
ESCMID	:	European Society of Clinical Microbiology and Infectious Diseases
FOX	:	Cefoxitin
GES	:	Guiana Extended Spectrum
GIM	:	German Imipenemase
HCWs	:	HealthCare Workers
ICU	:	Intensive Care Unit
IM	:	Inner Membrane
IMP	:	Inner Membrane Protein

IMPs : Imipenem Hydrolyzing Enzymes

KPC : Klebsiella Pneumonia Carbapenemase

MALDI- TOF : Matrix Assisted Laser Desorption/Ionization Time of Flight Mass Spectrometry

MATE : Multidrug and Toxic Compound Extrusion

MBLs : Metallo- β-Lactamases

MDR : Multi Drug Resistant

MDR- GNB : Multi Drug Resistant Gram Negative Bacilli

MFP : Membrane Fusion Protein

MFS : Major Facilitator Superfamily

MIC : Minimal Inhibitory Concentraion

MHT : Modified Hodge Test

MRSA : Methicillin Resistant Staphylococcus Aureus

NBC- A : Non- Metallo Carbapenemase of Class A

NDM : New Delhi Metallo beta Lactamases

OXA : Oxacillinase

OM : Outer Membrane

OMPF : Outer Membrane Protein F

PBPs : Penicillin Binding Proteins

PC : Penicillinases

PCR : Polymerase Chain Reaction

PL : Phospholipid

PG : Peptidoglycan

RND : Resistance Nodulation Cell- Division

SHV : *S*ulfhydryl *V*ariable

SPP. : Species

SMR : Small Multidrug Resistance

TEM	:	Named after the patient (Temoneira) providing the first sample
VAP	:	Ventilator Associated Pneumonia
VEB	:	Vietnam Extended Spectrum- β -Lactamases
VIM	:	Verona Integron-Encoded Metallo-β –Lactamase
ZnSo4	:	Zinc Sulphate

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Introduction

Acinetobacter baumannii has emerged as an important nosocomial pathogen in outbreaks of hospital infection and is ranked second after *Pseudomonas aeruginosa* among nosocomial pathogens of aerobic non fermentive Gram-negative bacilli **(Valentine et al., 2008**). Interest in this pathogen emerged about its capability of acquiring new mechanisms of resistance **(Kempf and Rolain., 2012).**

Acinetobacter baumannii infections are often difficult to eradicate due to high-level of resistance to many antibiotics, including (beta-lactams, aminoglycosides, chloramphenicol, tetracycline, and rifampin) as a result of both intrinsic and acquired mechanisms. Carbapenems (e.g., imipenem and meropenem) have become the drugs of choice against Acinetobacter infections in many centers but are being compromised by the emergence of carbapenem-hydrolyzing ß-lactamase (carbapenemase) **(Brown and Amyes, 2006).**

The resistance of *Acinetobacter baumannii* to carbapenems can be mediated by one of the resistance mechanisms that are known to occur in bacteria, including enzymatic inactivation, active efflux of drugs and modification of target sites. The production of carbapenem- hydrolyzing ß-lactamases (carbapenemases) is the most common mechanism responsible for carbapenem resistance in *Acinetobacter baumannii* **(Chihi et al., 2012).**

Oxacillin hydrolyzing β –lactamases (class D) are mostly described in *Enterobacteria, Acinetobacter baumannii, Pseudomonas aeruginosa* and *Klebsiella pneumonia*. *OXA* enzymes are resistant to penicillins, cloxacillin, oxacillin and methicillin, in certain cases, they also confer resistance to cefepime and to carbapenems **(Jan and Niels., 2006).** The commonest resistance is the production of oxacillinases encoded by genes of the *OXA-23* lineage, which may be plasmid or chromosomally located **(Chihi et al., 2012).**

OXA-23 carbapenemase-producing *Acinetobacter baumannii* are becoming increasingly widespread, with reports from Europe, South America, or Asia. In 2002, 49 strains of imipenem resistant *Acinetobacter baumannii* producing the carbapenenase *OXA-23* were isolated in South Africa. Many strains of *OXA-23* producing *Acinetobacter baumannii* from the same clone were responsible for an epidemic of nosocomial infection from 2005 to 2007 in Tunisia (**Andriamanantena et al., 2010).**

Wide spread of *Acinetobacter* infections and difficulty in treatment make an urgent to early, sensitive, and accurate methodology to detect causes of resistance and effective line in management. The phenotypic detection of carbapenemase producing isolates, can be tested by the modified Hodge test (MHT) test, as it indicates carbapenemase production by the test microorganism **(Noyal et al., 2009).**

Phenotype- based techniques for identifying in vitro production of carbapenemases, such as MHT, are not highly sensitive and specific **(Nordmann et al., 2012).** Currently, there is no standardized direct phenotypic method for the detection of *Acinetobacter baumannii* carbapenemases in routine microbiological laboratories, although there are indirect methods that are based on the ability of some compounds to inhibit carbapenemases. Polymerase chain reaction (PCR)- based methods remain the optimal tool for identification of *OXA* type carbapenemases **(Kempf et al., 2012a).**

An infection control strategy and restrictions in the use of carbapenem should be implemented in order to reduce the incidence of infection by such resistant strains **(Perez et al., 2007).**

Aim of work

The aim of this work is to detect *OXA-23* gene in carbapenem resistant *Acinetobacter baumannii* species, using PCR.

Chapter I

Acinetobacter Species

Acinetobacter species are gram- negative bacteria that have become one of the most difficult pathogens to treat. The species Acinetobacter baumannii, largely unknown 30 years ago, has risen to prominence particularly because of its ability to cause infections in immunocompromised patients ***(Evans et al., 2013).***

These bacteria survive for a long time in the hospital environment and thereby the apportunities for cross infection between patients are enhanced ***(Karthika et al., 2009).***

Taxonomy :

Historical Taxonomy

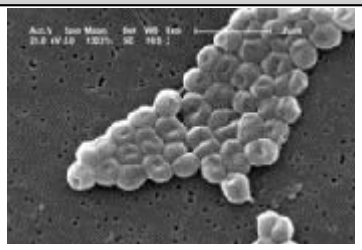
Bacteria classified as members of the genus Acinetobacter have suffered a long history of taxonomic change. The original concept of the genus Acinetobacter included a heterogenus collection of nonmotile, Gram-negative, oxidase-positive, and oxidase-negative saprophytes that could be distinguished from other bacteria by their lack of pigmentation ***(InGram and Shewan, 1960).***

The subcommittee on the taxonomy of moraxella and allied bacteria recommended that the genus Acinetobacter comprise only oxidase-negative strains ***(Lessel, 1971).***

Current Taxonomy

The genus *Acinetobacter*, as currently defined, comprises Gram-negative, strictly aerobic, nonfermenting, nonfastidious, nonmotile, catalase-positive, oxidase-negative bacteria with a DNA G+C content of 39% to 47%. Based on more recent taxonomic data, it was proposed that members of the genus *Acinetobacter* should be classified in the new family *Moraxellaceae* within the order *Gammaproteobacteria*, which includes the genera *Moraxella*, *Acinetobacter*, *Psychrobacter*, and related organisms (table 1) ***(Mugnier et al., 2008).***

Table (1): Scientific classification of Acinetobacter.

Acinetobacter	
	*Acinetobacter*
Scientific classification	
kingdom:	Bacteria
phylum:	Proteobacteria
class:	Gamma Proteobacteria
order:	Pseudomonadles
Family:	Moraxellaceae
Genus:	***Acinetobacter***
Species	
A. baumannii A. calcoaceticus A. lwoffii A. ursingii A. haemolyticus A. junii A. johnsonii A. radioresistens A. ursingii A. schindler	

(Mugnier et al., 2008).

Morphology:

The Gram- stain morphology of isolate that belong to the genus Acinetobacter are Gram-negative coccobacillary cells often appearing as diplo-cocci (Fig.1). This similarity in appearance to Neisseria can be rapidly distinguished by oxidase test, Acinetobacter is oxidase negative. Laboratory workers should also be aware of the *fact that* Acinetobacter species may initially appear as Gram-positive cocci in direct smears of clinical specimens and in smears prepared from positive blood-culture bottles ***(Niumsup et al., 2009).***

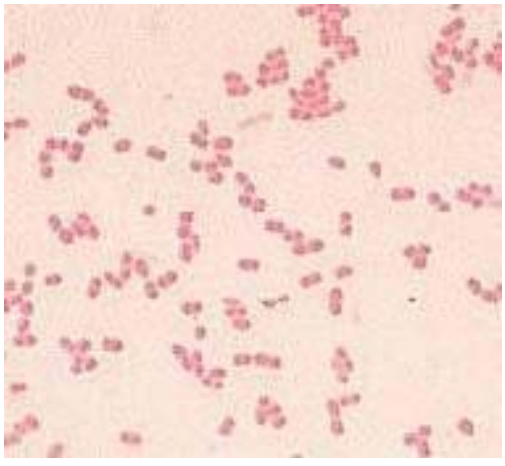


Fig. (1): The Gram-stain morphology of isolate that belong to the genus Acinetobacter. They are Gram-negative coccobacillary cells often appearing as diplo-cocci ***(Niumsup et al., 2009).***

Culture Characteristics:

Acinetobacter species of human origin grow well on solid media that are routinely used in clinical microbiology laboratories, such as sheep blood agar, at 37°C incubation. After 24 to 48 hours of growth on blood agar, the colonies are between 0.5 and 2mm in diameter, translucent to opaque (never pigmented), convex, and entire. Most strains grow well on MacConkey agar and produce a faint pink tint. Certain glucose-oxidizing Acinetobacter may also cause a unique brown discoloration of heart infusion agar with tyrosine or blood agar into which glucose is incorporated ***(Fournier and Richet, 2006).***

Differential and selective media (minimal salt acetate) have been described for isolation of Acinetobacter spp. from contaminated