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Phenotypic detection of Carbapenem Resistant *Enterobacteriaceae* isolates and assessment of their susceptibility to the novel Ceftazidime-Avibactam combination.

Thesis

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أَعُوذُ بِاللَّهِ مِنَ الشَّيْطَانِ الرَّجِيمِ

بِسْمِ اللَّهِ الرَّحْمَٰنِ الرَّحِيمِ

قَالُوا سُبْحَانَكَ لَا عِلْمَ لَنَا

إِلَّا مَا عَلَّمْتَنَا ۖ إِنَّكَ أَنْتَ الْعَلِيمُ الْحَكِيمُ

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Dalia Mohamed Ahmed Ibrahim

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List of Abbreviations

Abbr.	Full-term

AK : Amikacin.

AM : Ampicillin.

AMC : Amoxicillin / calvulinic acid.

ATM : Aztreonam.

ATP : Adenosine triphosphate.

CAZ : Ceftazidime.

CDC : The Center for Disease Control and Prevention.

CDT : Combined Disc Test.

CIP : Ciprofloxacin.

CLED : Cystine-Lactose-Electrolyte-Deficient-medium.

CLSI : Clinical and Laboratory Standards Institute.

CMS : Colistin methane sulphonate.

CP : Contact Precaution.

CRE : Carbapenem-resistant *Enterobacteriaceae*.

CSU : Catheter-stream urine.

CTX : Cefotaxime.

EDTA : Ethylene diamine tetraacetic acid.

ESBL : Extended-spectrum β-lactamases.

ETA : Endotracheal aspirate.

FEP : Cefepime.

FOX : Cefoxitine.

GN : Gentamycin.

HICPAC: The Healthcare Infection Control Practices Advisory Committee.

ICU : Intensive care units.

IMP : Imipenemase.

IV : Intravenous

KPC : *Klebsiella pneumoniae* carbapenemase.

LPS : Lipopolysaccharide.

MBLs : Metallo-beta-lactamases.

mCIM : Modified carbapenem inactivation method.

MDR : Multidrug resistant.

MEM : Meropenem.

MHA : Muller Hinton agar.

MHT : Modified Hodge test.

MIC : Minimal inhibitory concentration.

MSU : Mid-stream urine samples.

NDM : New Delhi metallo- β-lactamase.

NPV : Negative predictive values.

OMP : Outer membrane porines.

OXA : Oxacillinases.

PBPs : Penicillin-binding proteins.

PDR : Pandrug-resistant.

PPV : Positive predictive values.

SD : Standard deviation.

SHEA : The Society for Healthcare Epidemiology of America

SPSS : Statistical package for Social Science.

SXT : Sulphamethoxazole / Trimethoprim.

TPZ : Piperacillin / Tazobactam.

US FDA: United States Food and Drug Administration.

UV : Ultraviolet.

VAP : Ventilator-associated pneumonia.

VIM : Verona integron metallo- β-lactamase.

WHO : World Heath Organization.

XDR : Extensively-drug resistant.

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Introduction

Enterobacteriaceae are common causes of both community-acquired and hospital acquired infections including urinary tract, bloodstream, and lower respiratory tract infections ***(Wang , et al. 2015)***.

Dissemination of infection by extended-spectrum β-lactamases (ESBL) and AmpC β lactamases producing *Enterobacteriaceae* has led to increased use of carbapenems and the emergence of carbapenem-resistant *Enterobacteriaceae* ***(Baran and Aksu , 2016)***.

The mechanisms underlying carbapenem resistance in *Enterobacteriaceae* are complex and include both the production of carbapenem hydrolyzing lactamases (carbapenemase-producing CRE [CP-CRE]) and resistance due to the presence of a combination of other factors (non-CP-CRE), such as hyperproduction of AmpC lactamases or ESBLs combined with altered membrane permeability ***(Pierce et al., 2018)***.

The resistance among *Enterobacteriaceae* represents a major public health problem and has become a leading cause of morbidity and mortality worldwide ***(Goodlet et al., 2016)***. Their identification is of primary importance for the choice of appropriate therapeutic schemes and the implementation of proper infection control measures ***(HrabaÂk et al., 2014)***.

To prevent spread of carbapenemase producers, rapid detection of these bacteria has become imperative ***(Nordmann, 2014)***. A new straight forward inexpensive phenotypic test called mCIM was developed to detect carbapenemase production in *Enterobacteriaceae* ***(Pierce et al., 2017)***. It is currently recommended by Clinical and Laboratory Standards Institute (CLSI) for detection of carbapenemase among *Enterobacteriaceae* clinical isolates. ***(Yu et al., 2018)***.

This method showed high concordance with results obtained by PCR to detect genes coding for the carbapenemases KPC, NDM, OXA-48, VIM, IMP and OXA-23 ***(Nordmann et al., 2012)***.

Emergence of *enterobactericeae* producing carbapenemases resulting in broad resistance to most beta lactam antibiotics including last line carbapenems ***(Morrill et al., 2015)***.

Ceftazidime-avibactam is the combination of the established third-generation cephalosporin ceftazidime, with the novel non-β-lactam β- lactamase inhibitor avibactam ***(Li et al., 2015)***. Avibactam inhibits a broad range of serine β-lactamases including Ambler class A (ESBL and KPC), class C (AmpC) and some class D (OXA-48) enzymes ***(Ehmann et al., 2013)***. In combination with ceftazidime, avibactam restores activity of ceftazidime against a number of clinically relevant β-lactamase-producing Gram-negative pathogens causing serious infections ***(EUCAST, 2017)***.

Aim of the Work

The aim of this study was to evaluate the mCIM as a new method for phenotypic detection of carbapenemase-producing CRE and to test the in vitro susceptibility of the isolates to ceftazidime-avibactam .

I. Enterobacteriaceae

The *Enterobacteriaceae* is a very large family of Gram-negative bacilli that are similar in morphology and cultural characters. More than 130 different species of *Enterobacteriaceae* exist in 32 different genera. This family includes, *Escherichia, Salmonella, Shigella, Klebsiella, Citrobacter, Proteus, Yersinia and Serratia* ***(Kayser, 2005)***. Members of this family can be differentiated from each other by biochemical reactions and antigenic structure ***(Cullimore, 2000)***.

General characteristics:

They are non-spore forming, facultative anaerobic rods or coccobacilli ranging from 0.3 to 1.0 mm long, motile by means of peritricate flagella except *Klebsiella and Shigella* spp that are non-motile ***(Abbott, 2007)***.

They are oxidase negative, catalase positive, ferment glucose to produce lactic acid only or lactic acid with gas, reduce nitrate to nitrite, can grow on ordinary media as well as on selective and differential media e.g. MacConkey agar ***(Ananthanarayan and Paniker, 2006).***

Classification:

1. **According to lactose fermentation *(Janda and Abbott, 2008)***:
    - ***a)*** **Lactose fermenter: e.g.** *E.coli, Klebsiella, and Citrobacter.*
    - ***b)*** **Lactose non-fermenter: e.g.** *Salmonella, Shigella, Proteus and Yersinia.*
    - ***c)*** **Late lactose fermenter: e.g.** *Shigella sonnei.*

2. **According to pathogenicity:**

a) True pathogens *(Levinson, 2006)*:

Primary pathogenic strains are *Salmonella, Shigella, and Yersinia* species.

- Typhoid fever and enterocolitis are caused by *Salmonella* species.
- Bacillary dysentry is caused by *Shigella* species**.**
- Plague, enterocolitis and mesenteric adenitis are caused by *Yersinia* species.

b) Commensals:

Most of *Enterobacteriaceae*, such as *E.coli* are part of gut flora and do not cause disease exept in case of inadequate host defenses or when they reach tissues outside their natural habitat ***(Carrol and Hobden, 2010)***.