INTRODUCTION

The growing increase in the rates of antibiotic resistance is a major cause for concern in *Enterobacteriaceae* family. β-Lactams have been the mainstay of treatment for serious infections, and the most active of these are the carbapenems**.** Carbapenem resistance among *Enterobacteriaceae,* in particular *Escherichia coli (E.coli)* and *Klebsiella pneumoniae (K. pneumoniae)* is an emerging problem worldwide. Several resistance mechanisms have been reported to circumvent the efficacy of carbapenems, and carbapenemases (carbapenem-hydrolyzing β-lactamases) are the most prominent enzymes that neutralize carbapenems ***(Piotr et al., 2014).***

The real prevalence of carbapenemase-producing *Enterobacteriaceae* remains unknown because many countries worldwide do not report rates of antibiotic susceptibility ***(Voulgari et al., 2013).***

A large variety of carbapenemases belonging to three molecular classes of β-lactamases has been identified in *Enterobacteriaceae:* the Ambler class A e.g (KPC), class B e.g (NDM and IMP) and class D e.g (OXA 48) β-lactamases ***(Nordmann and Poirel, 2013).***

The OXA-48 is an active-serine-site enzyme like Ambler class A and class C β-lactamases, differing from class A and C enzymes in amino acid structure and is usually not inhibited by

clavulanic acid, tazobactam, and sulbactam. It was first identified from a carbapenem-resistant *Klebsiella pneumoniae* isolate that had been recovered in Istanbul, Turkey, in 2001. Occurrence of OXA-48 producers has also been reported in Africa, most of the data are from the northern countries ***(Leila and Rémy, 2014). Galler et al. (2013)*** found that OXA-48 producer isolate recovered by them, was multidrug resistant and exhibited a high level of resistance to all β-lactams, including broad-spectrum cephalosporins, cephamycins, monobactams and carbapenems.

The worldwide spread of *Enterobacteriaceae* expressing carbapenemases now represents a significant threat for the public health and requires efforts toward detection and infection control strategies. The early identification of carbapenemase-producing strains in clinical infections is mandatory to prevent the development of difficult or impossible-to treat infections ***(Leila and Rémy, 2014).***

Analysis of carbapenem hydrolysis with modified Hodge test or the Carba tests are methods that can be used to confirm carbapenemase-production. However, these classical methods are slow and have so far been evaluated in only a few laboratories, and their performance in laboratories lacking extensive experience with β-lactamase detection remains to be determined. It is not recommended to use the modified cloverleaf (Hodge) test as its results are difficult to interpret and sensitivity and specificity are poor ***(André et al., 2012)***.

Carbapenemase gene detection by molecular methods is the gold standard but is available in only a few reference laboratories, and phenotypic tests have therefore been developed ***(Anneke et al., 2014).***

AIM OF THE WORK

- To detect OXA-48 gene in *Enterobacteriaceae* by using Mastdisks inhibitor combinations disks as a phenotypic method and PCR as a molecular method and to evaluate the accuracy of clinical use of phenotypic method on clinical isolates as a rapid and easy method.
- To identify OXA-48 gene prevelance in carbapenem resistant isolates in comparison to other genes responsible for other carbapenamase enzymes (KPC and NDM).

BETA-LACTAM ANTIBIOTICS

Beta-lactam antibiotics are large number of natural, semisynthetic and synthetic antibiotics. They are applied in treatment of bacterial infections and subdivided into different structural subtypes illustrated in table (1).

Table (1): Groups and examples of β -lactam antimicrobial agents *(Formulary, 2007).*

β-lactam groups	Examples of antimicrobial agents
Penicillins	▪ Penicillin G, penicillin
	▪ Penicillinase resistant penicillins: methicillin, nafcillin, oxacillin, cloxacillin
	▪ Aminopenicillins: ampicillin, amoxicillin
	▪ Carboxypenicillins: carbenicillin, ticarcillin
	▪ Ureidopenicillins: mezlocillin, piperacillin
Cephalosporins	▪ First generation: cefazolin, cephalothin, cephalexin
	▪ Second generation: cefuroxime, cefaclor, cefamandole, cefamycins (cefotetan, cefoxitin)
	▪ Third generation: cefotaxime, ceftriaxone, cefpodoxime, ceftizoxime, cefoperazone, ceftazidime
	▪ Fourth generation: cefepime, cefpirome
Carbapenems	▪ Imipenem, meropenem, ertapenem,doripenem
Monobactams	▪ Aztreonam

Carbapenems

Carbapenems are β-lactam agents that are structurally like penicillins but with substitution of carbon atom for sulfur atom at position 1 and so are called carbapenams ***(Dortet et al., 2012).***

Carbapenems are used in hospitals for serious infections caused by many forms of bacteria except intracellular bacteria. They are administerated intravenously due to poor oral bioavailability. They include imipenem, meropenem, ertapenem and doripenem ***(Piotr et al., 2014).***

Mechanism of action:

They enter the bacterial cell wall of Gram negative bacteria through porins, bind and act as inhibitors of the peptidase domain of different penicillin binding proteins (PBPs) and that leads to autolysis of these proteins ***(Scheetz et al., 2007).***

Clinically available carbapenems:

1-Imipenem and cilastatin

Imipenem (IPM) was the first developed carbapenem in 1985. Usually IPM is combined with cilastatin (an inhibitor of dehydropeptidase-1) to decrease it's rapid hydrolysis in renal tubular cells. It is used in treatment of urinary tract infections, bacterial septicemia and lower respiratory tract infections ***(Scheetz et al., 2007).***

2- Meropenem

- Meropenem (MPM), released in 1992, was found to be more potent against Gram negative bacteria compared to IPM. It is stable to dehydropeptidase-1enzyme and therefore can be

given without cilastatin. It is used in treatment of infections like meningitis and pneumonia ***(Chan et al., 2007).***

3- Ertapenem

- Ertapenem exhibits microbiologic activity similar to meropenem however, it shows poor activity against *Acinetobacter and Pseudomonas aeruginosa* ***(Lesho et al., 2005).***
- Risk factors for ertapenem resistant *Enterobacteriaceae* infection include long stay intensive care unit stay,the use of central venous catheter, the use of mechanical ventilation receipt and the exposure to any antibiotic during the 30 days prior to a positive culture result of these organisms ***(Lesho et al., 2005).***

4-Doripenem

Doripenem is the latest released carbapenem to be released (2001). Unlike imipenem it escapes hydrolysis in renal tubules by dehydropeptidase-1 due to β-methyl group. It has a weak convulsive effect compared to other carbapenems. It is used in treatment of urinary tract infections and intra-abdominal infections ***(Kathryn et al., 2013).***

Mechanism of Carbapenem Resistance

Carbapenem resistance among *Enterobacteriaceae* is an emerging problem worldwide. The main mechanisms by which microorganisms show resistance to carbapenems are modifications

to outer membrane permeability which leads to loss of porin that mediates resistance and up-regulation of efflux systems ***(Francis et al., 2012).*** Changes in penicillin-binding proteins have also been implicated in carbapenem resistance and production of specific carbapenem-hydrolysing β lactamases (carbapenemases) (figure 1) ***(Sheng et al., 2012).***

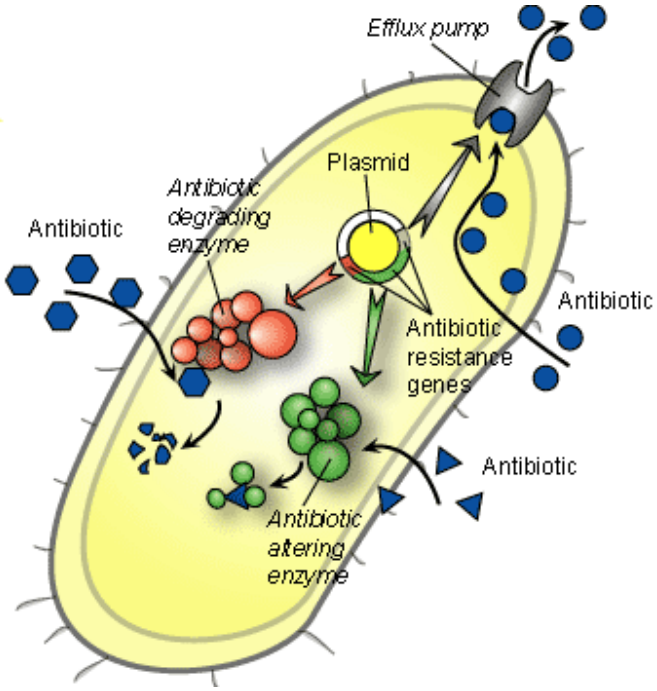

Figure (1): Mechanisms of resistance against carbapenems *(Sheng et al., 2012)*.

Mechanisms of Antibiotic Resistance in Enterobacteriaceae

The emergence and spread of antimicrobial resistance continue to challenge the our abilities to treat serious infections in both the nosocomial and the community settings. While new antimicrobial agents were designed to treat multi-resistant pathogens that have been introduced in the past few years, resistance has continued to emerge and spread ***(Piotr et al., 2014).***

- ***Causes of Spread of Antibiotic Resistance:***

The tendency for antibiotic use to promote the emergence of resistant pathogens is called antibiotic pressure. Antibiotic resistance emerges more in hospitals, and hospital pathogens tend to be more resistant than in community pathogens.Self-medication with antibiotics, overuse and misuse of antibiotics by doctors and patients have caused the occurance of antimicrobial resistance ***(Huang et al., 2013).***

In addition, the mechanisms of acquired bacterial resistance are accelerated when encoded on transferrable plasmids and transposons ***(Huang et al., 2013).***

- ***Mechanisms of Resistance:***

In Enterobacteriaceae, decreasing the porin production or increasing the expression of efflux pump systems leads to modification in membrane permeability. The combination of

these phenomena with modification of the drug targets in various clinical isolates emerge a Multi Drug Resistant phenotype (MDR) ***(Day et al., 2013).***

Antibiotic resistance can be divided into natural resistance and acquired resistance.

I. Natural or intrinsic resistance

Natural resistance means that the bacteria are 'intrinsically' resistant. Intrinsic resistance was believed to result primarily from low outer membrane permeability. However, it is now known to be the result of the combined action of multidrug resistance pumps and decreased outer membrane permeability ***(EUCAST, 2013).***

β-lactam antibiotics usually target penicillin-binding proteins (PBPs), in the cytoplasmic membrane and β-lactamases in the periplasmic space of gram negative bacteria. Once antibiotics bind to PBPs, they are actively expelled through efflux pumps ***(Huang et al., 2013).*** The relation between porin channels and efflux pump system is shown in figure (2)**.**

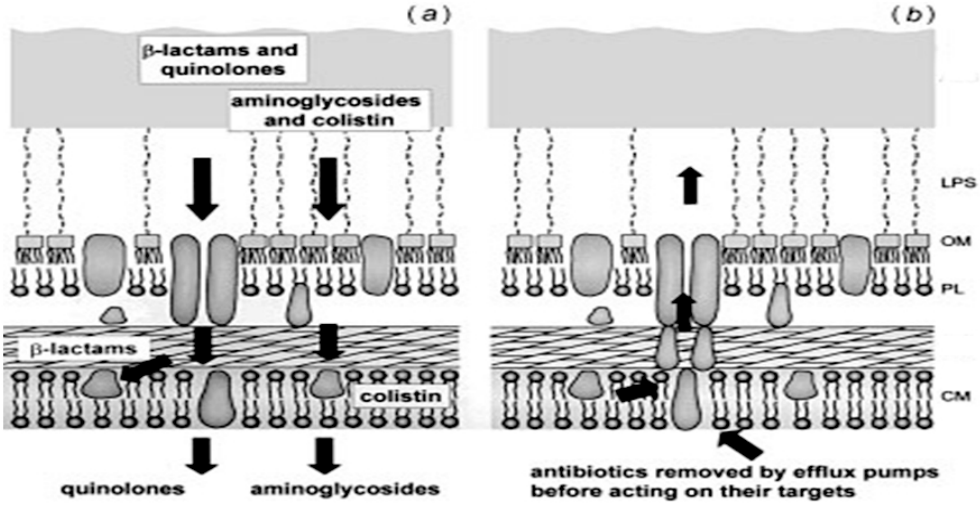

Figure (2): Efflux systems reverse the diffusion of antibiotics across the OM. (The efflux pumps comprise three components: an energy-dependent pump in the CM, a porin in the OM and an adapter protein joining the two membrane components. Antibiotics which have entered the cell are collected from the cytoplasm, the cytoplasmic membrane or the periplasm and expelled from the cells through the porin) ***(Huang et al., 2013)***.

(A) Efflux Pumps

The gram-negative bacterial envelope consistes of influx pumps for nutrients and efflux pumps for toxic compounds. Among these transporters are distinct energy dependent efflux pumps that recognize toxic agents such as antibiotics and extrude (pump out) the agent from the periplasm/cytoplasm to the exterior (environment) of the cell ***(EUCAST, 2013).***

The basic structure of these pumps comprises a transporter in the inner membrane, a periplasmic membrane fusion protein and a channel in outer membrane. These pumps provide a means of multidrug resistance (MDR) phenotype to quinolones, macrolides, tetracyclines ***(EUCAST, 2013).***

(B) Porin Loss

Porins channels are responsible for diffusion of β-lactam antibiotics e.g Omp K35 and Omp K36 found in *K.pneumoniae.* Loss of major porins in clinical isolates leads to high resistance to tetracyclines, chloramphenicol, quinolones, aminoglycosides and β-lactam antibiotics ***(Huang et al., 2013).***

(C) Biofilms

Biofilms are microbial aggregations adhering to biological or non-biological surfaces. They represent a protected mode of microbial survival and growth in hostile environments. Biofilm infections are a huge problem in the clinic and cause many deaths and high health costs ***(Hölscher et al., 2015).***

E. coli and Klebsiella are the top two species causing urinary tract infection and are generally excellent biofilm formers. The eradication of *E.coli* biofilms required 220 times higher antibiotic concentrations than for the same strain in serum ***(Hölscher et al., 2015).***

II. Acquired resistance

Acquired resistance refers to bacteria that are usually sensitive to antibiotics but are liable to develop resistance. It is caused by mutation of cellular genes, aquisition of new genetic material such as plasmids or transposons which carry the

antibiotic resistant genes or inactivation of drug by enzymes ***(Jessica et al., 2015).***

(A) Mutation of cellular genes

Errors in the process of replication, insertion or deletion of DNA segments can cause a nucleotide sequence permanent change of the genome of an organism and lead to mutations ***(Liam et al., 2014).***

Mutation causes alteration of outer membrane permeability either by down regulation of porin genes so normally susceptible populations of bacteria may become resistant to antimicrobial agents or by over expression of efflux pump that extrudes the antibacterial agent from the cell before it can reach its target site and exert its effect ***(Jessica et al., 2015).***

(B) Aquisition of new genetic material

This occurs when DNA genetic material are transferred between the same species or different species of individual bacteria by a horizontal gene transfer (HGT) process. Conjugation, transduction and transformation are the mechanisms of HGT ***(Liam et al., 2014).***

1. **Conjugation:** occurs between nearby bacterial cells through plasmids transfer and is considered the main mechanism of HGT which occurs in *E.coli and Klebsiella* ***(Xu et al., 2014).***

2. Transduction: occurs when DNA is transferred between closely related bacterial cells through bacteriophages ***(Xu et al., 2014).***

3. Transformation: Bacterial transformation can be referred to as a stable genetic change to increase DNA quantity by uptake of DNA without associated proteins or cells (naked DNA).There are two forms of transformation and competence: natural and artificial ***(Jessica et al., 2015).***

(C) Inactivation of drug by enzymes (β-lactamases)

BETA-LACTAMASES AND CARBAPENEMASES

Beta-lactamases

β-lactamases are enzymes produced by many forms of bacteria and have the ability to inactivate pencillins and cephalosporins by opening their β- lactam ring 'neutralizing β-lactam antibiotics'. So, bacteria which produce these enzymes become resistant to traditional penicillin and penicillin based antibiotics (Figure 3) ***(Bassetti et al., 2011).***

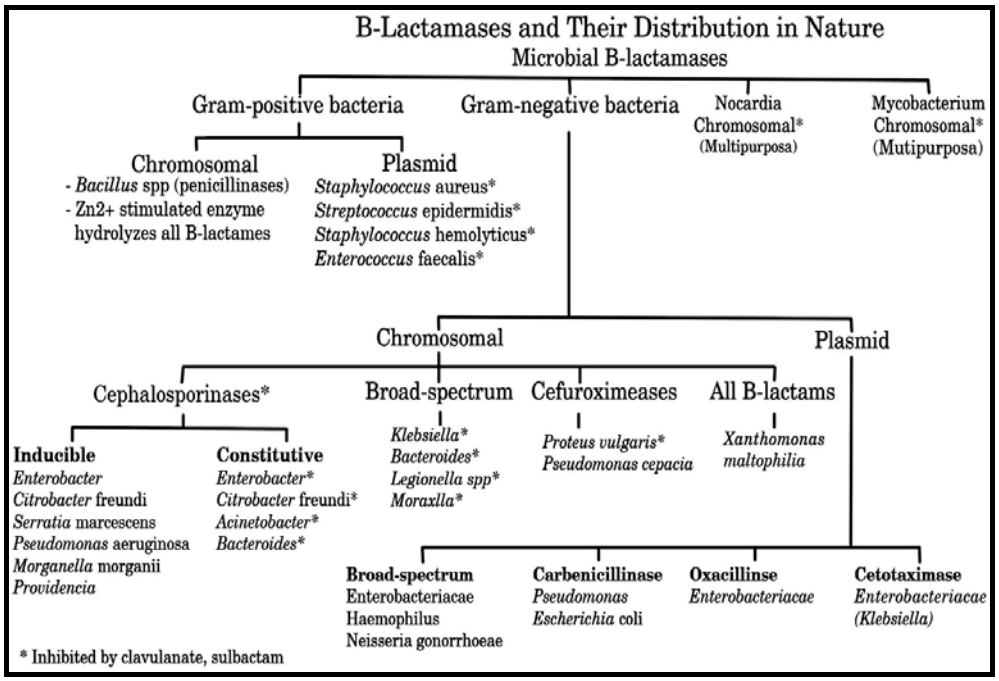

Figure (3): β-lactamases found in bacteria and their classification and synthesis, whether chromosomally or plasmid mediated *(Bassetti et al., 2011).*