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Carbapenem Resistance In Enterobacteriaceae

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Abstract

Carbapenem resistance is now a public health concern worldwide. Carbapenem is considered the last resort antimicrobial drug used for treatment of hospital care-associated infection and community-acquired infection that resistant to all other β-lactam drugs. There are different mechanisms by which bacteria become resistant to carbapenem drug including efflux pump, porin mutation and production of carbapenemase enzyme that hydrolyze the drug. Carbapenemase enzymes were identified largely in different members of the Enterobacteriaceae family which is a gram-negative bacteria responsible for a variety of infectious diseases and this was associated with increased morbidity and mortality rate worldwide. Many Risk factors were found to be associated with increased susceptibility to develop carbapenem resistance that should be searched for to prevent further spread of resistance. Various phenotypic and genotypic tests are used to detect carbapenemase production with different sensitivity and specificity. The current state of carbapenem resistance is well identified in many parts of the world while in other places such as sub-Saharan Africa, this is not well known.

Keywords: carbapenem,resistance, phenotypic test,

Introduction

Carbapenem-resistant Enterobacteriaceae (CRE) is now a public health problem worldwide **(1).** In the early 1990s., the first discovered carbapenem resistance-in Enterobacte-riaceae was published and in 2001, the first Klebsiella pneumoniae carbapenemase (KPC) producing Enterobact-eriaceae was reported. Thereafter, CRE has disseminated globally **(2).**

Carbapenems

Carbapenems are bactericidal β-lactam antibiotics used in the management of severe infections caused by bacteria that produce extended-spectrum β-lactamase with high efficacy **(3).** Meropenem, imipenem, ertapenem and doripenem are a few examples of carbapenem in use worldwide. Recently several mechanisms of resist-ance emerged as β-lactamases that hydrolyze carbapenem leaving narrow therapeutic options **(4).**

Mechanism of action

The β-lactam family of antibiotics that includes; penicillins, cephalosporins, monobactam and carbapenems shares common structure and mechanism of action. After entering through porins to the periplasmic space they inhibit transpeptidases. These enzymes enhance peptide cross-links during the synthesis of the cell wall. The similarity of β-lactam toD-alanyl-D-alanine (a res-

id-ue used in the formation of peptidoglycan) facilitate their binding to the transpeptidases. Carbapenem binds to transpeptidases, which causes them to lose their catalytic activity. This inhibits peptidoglycan polymers synth-esis by inhibiting the formation of cross-links between them that causes a build-up of new peptidoglycan precu-rsors without cross-linkages. This weak peptidoglycan in addition to the continued activity of autolysins, which destroys peptide bonds of peptid-oglycan, disturbs the cell wall and causes osmotic rupture of the cell **(5).**

Carbapenem resistannce Enterobacteriaceae (CRE)

CRE are Gram-negative bacteria resist carbapenem antibiotics that considered the drugs of last resort by producing enzyme called carbapenemase that hydrolyzes the antibiotic molecule. Experts have referred to CRE as "nightmare bacteria **(6).**

In 2013, CDC report that half of the patients who get bloodstream infections can be killed up by these bacteria **(7)**

Enterobacter cloacae (E. cloacae), as well as other Enterobacteriaceae, is a commensal organism present in the intestine. Infections caused by E. cloacae arranged as the third among all the Enterobacteriaceae **(8).**

Chromosome mediated AmpC β-lactamase is produced by Enterobacter cloacae and Enterobacter cloacae have resistance to amoxicillin/clavulanic, ampicillin, first and second-generation cephalosporin and cephamycin. So, carbapenems are used in the treatment that leads to the emergence of multi-drug resistance rapidly under antibiotic selection pressure. In many countries such as India, Australia, Spain, China and the United States**,** carbapenem-resistant E. cloacae infections have been documented **(9, 10, 11).**

The main mechanisms for carbapenem resistance in E. cloacae is a decrease in membrane permeability and producing carbapenemases. Also, combinations of either AmpC or ESBL and mutation of porins are other important mechanisms **(12).**

Mechanisms of carbapenem resistance:

The 3 main mechanisms for carbapenem resistance are efflux pumps, porin mutations and enzyme production **(13).**

1. Efflux pumps that actively transport carbapenem outside of the cell wall have been observed in resistant bacteria **(14).**
2. Porins mutation or loss of it that prevents the entrance of carbapenem into the periplasm space **(15).**
3. Carbapenemases enzymes which are a form of β-lactamase, destroy the β-lactam ring which is an important component of β-lactam drugs that bound to transpeptidases, is produced by Enterobacteriaceae. Carbap-enemases are classified according to the structure of the enzyme and the mechanism of the β-lactam hydrolysis into several classes **(16).**

In general there are two subgroups for CRE: carbapenemase-producing CRE (CP-CRE) and non-carbapenemase-producing CRE (non-CP-CRE) (Figure 1) **(17).**

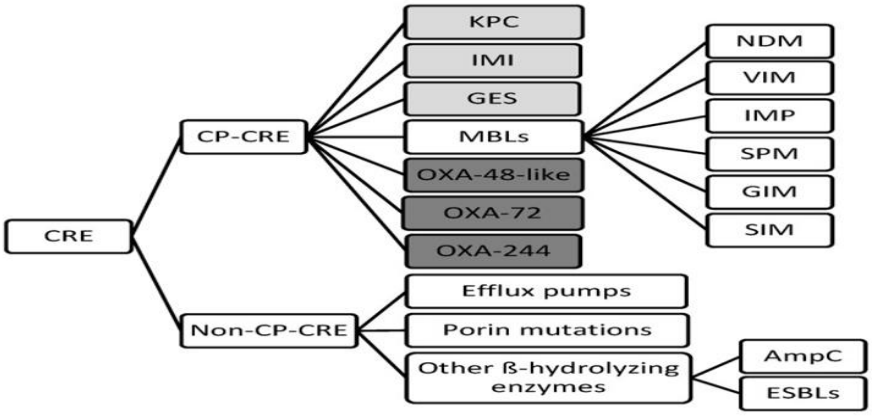

Figure 1: Classification of carbapenem resistance mechanisms in CRE.

AmpC : Type C ampicillinase ***** **CP** : Carbapenemase producing * **CRE** : Carbapenem-resistant Enterobacteriaceae *
ESBLs :Extended-spectrum ß-lactamase * GES :Guiana extended-spectrum ß-lactamase * **GIM** : German imipenemase * **IMI:** Imipenem-hydrolyzing ß-lactamase* IMP: Imipenem-resistant *Pseudomonas* carbapenemase*
KPC : *Klebsiella pneumoniae* carbapenemase*** MBLs :** Metallo-beta-lactamases *** NDM**: New Delhi metallo-β-lactamase*** OXA** : Oxacillinase *** SIM** : Seoul imipenemase * **SPM** : Sao Paulo metallo-ß-lactamase***VIM:** Verona integron-encoded metallo-β-lactamase

Classification of carbapenemases

Enzymes that cause carbapenem resistance are divided into three main classes according to Ambler classification **(18)** (Table 1).

Carbapenemase	KPC	MBLs (NDM, VIM, IMP)	OXA-48
Ambler class	A	B	D
Substrates of hydrolysis	whole β-lactams	whole β-lactams except for aztreonam	Penicillins and carbapenems
classic β-lactamase inhibitors	Minimally	No	No
Avibactam inhibition	Yes	No	Yes
Common species in Enterobacteriaceae	K.pneumoniae , E.coli, Enterobacter s pp.	NDM: K. pneumoniae, E. coli VIM: K. pneumoniae IMP: K. pneumonia	K. pneumonia

Table 1: Classification of common carbapenemases in Enterobacteriaceae.

Class A β-lactamases

These enzymes either chromosomally encoded as not metalloenzyme carbapenemase A (NmcA), Imipenem-hydrolysing β-lactamase (IMI), Serratia fonticola carbapenemase (SFC) and Serratia marcescens enzyme (SME), or plasmid encoded as Klebsiella pneumoniae carbapenemase (KPC), Guiana extended-spectrum (GES) and its derivative (GES-1 – GES-20), but all are partially inhibited by clavulanic acid **(19, 20)**. Class A carbapenemases are serine carbapenemases which mean that serine is required for hydrolysis of β-lactams that present at the active site of these enzymes **(21).**

In addition to K. pneumoniae isolates, other members of Enterobacteriaceae have been found to produce KPC including Klebsiella oxytoca, Escherichia coli, Proteus mirabilis, Salmonella enterica, Citrobacter freundii, Serratia marcescens and Enterobacter species (Ente-

robacter aerogens and Enterobacter cloacae) **(22, 23).**

Class B metallo-β-lactamases (MBL)

NDM (New Delhi Metallo-β-lactamase), GIM (German imipenemase), IMP carbapenemases (Imipenem-resistant Pseudomonas), SIM (Seoul imipenemase) and VIM (Verona integron-encoded Metallo-β-lactamase) are members of Metallo-β-lactamase families. These enzymes are encoded by genes present within integron structures and gene cassettes. Zinc at the active site of these enzymes is required for hydrolysis of carbapenem drugs hence, these enzymes can be inhibited by ethylenediaminetetraacetic acid (EDTA), a chelator of Zn2+ **(24).**

Class D β-lactamases

These carbapenemases include OXA enzyme type and the blaOXA genes are present on both chromosomes and plasmids. These enzymes are serine-β-lactamases and poorly inhibited by clavulanic acid and EDTA. In 1985, OXA β-lactamase was first described and the enzyme was isolated from patients in Scotland infected by Acinetobacter baumannii **(25).** There is wide geographic dissemination of OXA carbapenemase due to increased activity of OXA carbapenemases by upstream elements that control gene expression and also increase the strength of their promoters by insertions in the vicinity of these genes and hence increase resistance **(21).** OXA carbapenemases can mutate rapidly and they were reported frequently in the Enterobacteriaceae family which is a major public health problem worldwide **(26)**. The true prevalence rates of OXA-48 are difficult to estimate and OXA-48 producers are difficult to identify due to their point mutant analogs with extended-spectrum β-lactamase **(24).**

Risk factors:

Primary transmission places for CRE infections are hospital. Hospital admissions due to CRE from long-term acute care facilities (LTAC) or transferred from another hospital account for 75%. A significantly higher incidence of colonization and infection was found in patients admitted from LTAC facilities comparing with other hospitalized patients. A study in 2012, has been stated that over 30% of the patients with LTAC exposure was found to be colonized with CRE **(27, 28)**

High incidence and prevalence of CRE infections were reported in countries with antibiotic abuse where antibiotics are obtainable without a prescription. Because of limited antibiotic use in Japan, only 6.4% of healthy populations found to carry ESBL producing strains whereas in Thailand and Egypt, 58.4%, and 63.3% of the healthy population respectively were colonized because of the availability of antibiotics without prescription **(28).** Other Risk factors for CRE infection include mechanical ventilation, abuse of antibiotics, diabetes and compromised immune response Suboptimal maintenance practices includes inadequate cleaning and disinfection of medication cabinets, patient rooms, and medical equipment, that are used for both non-CRE patients and CRE are a cause of CRE **(29).** It has been found that exposure to different types of antibiotics (carbapenems, quinolones, glycopeptides, and β-lactams), organ/stem cell transplantation, mechanical ventilation, long time of hospitalization, ICU admission, surgery, urinary catheter, nasogastric catheter and central venous catheterization are risk factors for CRE **(30).** Decreases in the CRE rate was found in the hospital in which contact precaution for patients infected with Gram-negative bacilli was taken. Thus, it is important to find out the source of infection and patients who have any risk factor of infection and the main focus in treating patients at high

risk should be to prevent CRE infection **(31, 32).**

Identification of carbapenemases:
Many trials have been come out to detect the production of carbapenemases in bacteria. They divided into phenotypic and genotypic tests.

Phenotypic tests:

1. Modified Hodge Test
According to this test, the tenth dilution of 0.5 McFarland suspension of E Coli ATCC is inoculated on Mueller-Hinton agar plate and an imipenem disc(10-μg) is put in the center of the plate. Then the carbapenem-resistant strain is spreaded from the edge of the disc to the periphery of the plate therefore to form a straight line of inoculum. The plate is incubated overnight. Imipenem is hydrolyzed and the susceptible E. coli grow toward the disc, making a cloverleaf-like appearance if carbapenemase is released by the test strain**.** The MHT had been recommended as a confirmation test because of its high sensitivity and specificity in detecting carbapenemases**(33, 34).**

2. Carbapenem Inactivation Method
Firstly, a meropenem(10-μg) disc is incubated with the test strain for 2 hours at 35°C. Then, the meropenem disc is removed from the solution and put on a Mueller Hinton agar plate inoculated with sensitive E. coli strain. During the initial incubation the meropenem disc would have been inactivated, allowing for the growth of E. coli, making it appear as if the susceptible E. coli is resistant if carbapenemase is released but if no carbapenemase is released, an inhibition zone is formed**.** Because of its low cost and does not need special skills, the Clinical and Laboratory Standards Institute (CLSI) recommended this test as one of the confirmation tests of CPE. This method can be used for the detection of IMP, KPC, OXA-48 and Metallo-beta-lactamases such as NDM and VIM with sensitivity about 99% **(35, 36)**.

3. Combined disc Test
The chelating activity of ethylenediaminetetraacetic acid (EDTA) to zinc metal let it to be useful in detecting MBL enzymes as these enzymes need zinc for their activities**.** EDTA is added to a carbapenem disc, then is put on a plate inoculated with the suspected carbapenem resistance strain. A larger zone of inhibition around the EDTA/carbapenem disc compared with the carbapenem disc without EDTA indicates that MBL is present **(37, 38).**

4. Boronic Acid Inhibition Test
It is known that boronic acid compounds excellent inhibitors of class C β-lactamases**.** Recently, these compounds can be used for inhibition of class A carbapenemases. According to this test, 2-aminophenyl boronic acid (300 or 400 μg) is added to an ertapenem or meropenem disc. If KPC is present, there will be an increase in the zone of inhibition of 5 mm or greater compared with the ertapenem or meropenem disc alone **(39, 40).**

5. Carba NP test
This test is a rapid colorimetric test depend on the detection of pH changes that occur when imipenem is hydrolyzed by carbapenemases**.** The Carba NP test is highly sensitive in identifying MBL and KPC enzymes but difficulty identifying the activity of OXA-48 and GES-5. It is one of the phenotypic tests recommended by CLSI for the detection of carbapenemases **(41).**

Molecular tests

1. Polymerase Chain Reaction(PCR)
Various types of PCR as conventional, real-time, and multiplex PCR can be used for the detection of carbapenemase with high sensitivity and specificity **(42).**

2. Verigene

Verigene is a non-amplified test depends on nucleic acid extraction from positive blood cultures, microarray-based using capture and probes for detection. The process needs about 5 minutes for sample preparation and 2 hours for a run and so it is very useful in the rapid detection of various carbapenemase genes **(42, 43).**

3. BioFire FilmArray

It is an automated multiplex PCR that although firstly used for rapid detection of bacteremia and fungemia, now it can be used to detect the presence of carbapenemase genes as blaKPC. The sample preparation takes about 2-3 minutes and a turnaround time of 1 hour. It has 100% sensitivity and specificity in the detection of blaKPC but its cost is high which limits its use **(44).**

4. Gene Xpert

The detection of bla KPC, bla NDM, bla VIM, bla IMP, and bla OXA-48 can be achieved by the Xpert Carba-R. The sample preparation needs 1 minute and less than an hour for the run. The sensitivity and specificity of the Xpert Carba-R assay were higher compared with those of the reference culture and sequencing results **(45).**

5. **DNA Sequencing**

This method is used for the detection of carbapenemase genes and it can detect known and unknown genes that encode carbapenemases. In addition to that it can provide information about species and any other resistance genes. Because of its decreased cost, genome sequencing can now be applied in clinical microbiology laboratories. Metagenome sequencing uses DNA extracted directly from biological specimens instead of pure culture DNA which was used in whole-genome sequencing, **(46).**

Current resistance status

Although carbapenem resistance genes were documented among several countries in Asia, South America, and Europe, the situation in sub-Saharan Africa is not well documented **(47).**

The first strain of CRE was discovered in the 1980s after that it had been rapidly spread worldwide **(48).** Epidemiology studies suggest that there is a different geographic distribution for carbapenemases and specific carbapenemases prevalent in different parts of the world. For example, NDM-1 is prevalent in Pakistan and India, KPC is widespread in Italy, Greece, Colombia, Argentina, and United States while OXA-48 is endemic in North Africa, Turkey, the Middle-East and Malta **(49).**

Egypt is one of the first countries in the Eastern Mediterranean Region to establish a national surveillance system for health-care-associated infection (HAI). Egypt's HAI surveillance system that was established in May 2011, aimed to determine the prevalence rate and the incidence rate for HAI and to describe pathogens responsible for HAI to tell prevention and infection control activities **(50, 51).** There was an increase in the percentage of HAI cases due to CRE in Egypt between 2011 and 2017 **(52)** as from 3109 patients, 3836 Enterobacteriaceae isolates were reported to the surveillance system for HAI in Egypt and according to isolate-level analysis, from 2306 isolates that were delivered to the reference laboratory, 1105 with a percentage of 47.9% were CRE which mean that about half (47.9%) of the isolates were CRE **(52)**. This is higher than measures stated in other Arab, Asian or African countries **(53, 54, 55, 56**). The incidence of all CRE (HAI and non-HAI) in United States, Canada and China was 0.1–0.4/10,000 patient-days, 0.2 per 10,000 patient-days and 0.4 per 10,000 patient-days respectively which is much

lower than the incidence of CRE HAI reported from Egypt(3.7/10,000 patient-days) **(57, 58, 59).**

Therefore, it is important to implement strict infection control measures to prevent further dissemination of CRE in Egypt **(52).**

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