بسم الله الرحمن الرحيم

MONA MAGHRABY

شبكة المعلومات الجامعية

التوثيق الالكتروني والميكروفيلم

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شبكة المعلومات الجامعية

التوثيق الإلكتروني والميكروفيلم

جامعة عين شمس

التوثيق الإلكتروني والميكروفيلم

قسم

نقسم بالله العظيم أن المادة التي تم توثيقها وتسجيلها
علي هذه الأقراص المدمجة قد أعدت دون أية تغيرات

يجب أن

تحفظ هذه الأقراص المدمجة بعيدا عن الغبار

MONA MAGHRABY

INTRODUCTION

The carbapenemase-producing *Enterobacteriaceae* are among the most clinically significant multidrug-resistant bacteria. Its rise has led to an increase in the use of the polymixi B and colistin because of limited effective alternative antibiotic treatment ***(Kadri et al., 2015)*.**

Because these bacteria usually remain susceptible to polymyxins, an old class of antimicrobial drugs almost abandoned in the 1970s because of their potential toxicity, interest in polymyxins (colistin and polymyxin B) has been renewed worldwide ***(Falagas et al., 2005; Nordmann et al., 2011)*.**

The increasing use of colistin explains why acquired colistin resistance may now be added to the carbapenem resistance trait in *Enterobacteriaceae* ***(Monaco et al., 2014)*.**

In a study from the USA, 13% of 246 patients with carbapenem-resistant *Klebsiella pneumoniae* isolates were found to have colistin resistance, which was shown in that study to be associated with increased mortality ***(Rojas et al., 2017)***.

Acquired resistance to colistin in *Enterobacteriaceae* results mostly from modification of lipopolysaccharide**.** Addition of phospho-ethanolamine, 4-amino-l-arabinose cationic groups, or both to lipopolysaccharide decreases

polymyxin binding to the bacterial outer membrane. Addition of these groups may be associated with chromosome encoded mechanisms (mutations in Pmr AB or PhoPQ two-component systems or alterations of the *mgr B* gene) ***(Olaitan et al., 2014)*.**

Reports in the last few years revealed that addition of phospho-ethanolamine may also be plasmid mediated through the mcr-1 gene, which confers the first known plasmid-mediated resistance to colistin in isolates from humans and animals, the mcr-1 gene was identified in several plasmid backbones, mostly in *Escherichia coli* ***(Arcilla et al., 2016; Liu et al., 2016; Malhotra-Kumar et al., 2016; Poirel et al., 2016)*.**

The standard reference technique for determining susceptibility to polymyxins is broth microdilution, which requires fastidious attention and a long time (24 hr) to perform ***(Hindler and Humphries, 2013)*.**

Other techniques for determining susceptibility to polymyxins (disk diffusion and E-test) have been proposed and also provide results in 18–24 hr. Because of poor diffusion of polymyxin molecules in agar, rates of false susceptibility test results are high (up to 32%) ***(Tan and Ng, 2007; Hindler and Humphries, 2013)*.**

Rapid two hours assays that can be performed on clinical isolates have been developed recently based on the detection of acidic products from bacterial metabolism in the presence of

colistin or polymyxin B ***(Jayol et al., 2016; Nordmann et al., 2016b; Coppi et al., 2018; Poirel et al., 2018)***.

As an example rapid Polymyxin NP test was developed for the rapid detection of colistin-resistant *Enterobacteriaceae,* Rapid Polymyxin NP test relies on the colorimetric detection of rapid glucose metabolization associated with bacterial growth in the presence of a defined concertation of colistin ***(Nordmann et al., 2016b)***.

While these assays are sensitive and specific, they do not provide information on the mechanism of resistance, which may become important if *mcr-1* allele-specific inhibitors are introduced to the market ***(Daly et al., 2017)***.

Other phenotypic tests of colistin resistance based on inhibition of *mcr-1* activity by EDTA have been developed and may differentiate *mcr-1* from other mechanisms as colistin-MAC test ***(Esposito et al., 2017; Coppi et al., 2018)***.

AIM OF THE WORK

- Evaluation of the diagnostic efficacy of the rapid polymyxin NP test for the diagnosis of colistin-resistant *Enterobacteriacae*.
- Determination of the prevalence of *mcr-1* and *mcr- 2* genes among the colistin-resistant *Enterobacteriacae.*
- This was done within six months, in the main microbiology laboratory, clinical pathology department, Ain Shams University hospitals.

Chapter 1

ANTIBACTERIAL RESISTANCE

The use of antibiotics to treat bacterial infections is a key component of modern medicine but antibiotic resistance, particularly multi-resistance, in the *Enterobacteriaceae* is an increasing global problem, with strains resistant to most or even all available antibiotics emerging. Much of the resistance in *Enterobacteriaceae* is due to mobile genes captured from various source species by different mobile genetic elements and transferred to plasmids. However, mutations in chromosomal genes are also important in conferring or enhancing resistance to certain classes of antibiotics ***(Martinez et al., 2008)***.

COLISTIN

A. Mechanism of action of colistin

Colistin is a polycationic antibiotic, and has significant activity against Gram negative bacteria, such as *Enterobacteriaceae*. The outer cell membrane of Gram negative bacteria is the main site of action for colistin. When colistin binds to lipopolysaccharides in the outer membrane, electrostatic interaction occurr between the α,γ- di amino butyric acid of colistin and the phosphate groups of the lipid A region of lipo poly saccharide (LPS). It competitively displaces divalent cations (Ca2+ and Mg2+) from the phosphate groups of membrane lipids ***(Dixon and Chopra, 1986; Bialvaei and Samadi Kafil, 2015)***.

Therefore, disruption of LPS may cause increased permeability of the outer membrane and leakage of intracellular contents, ultimately leading to cell death ***(Li et al., 2006; Biswas et al., 2012)***.

Unfortunately, during the last few decades, the emergence of colistin-resistant isolates has been frequently reported ***(Yahav et al., 2012; Bialvaei et al., 2015)***.

The increased inappropriate use of this drug, especially as mono therapy could be the cause of this problem ***(Lee and Ko, 2014; Bialvaei et al., 2016)***.

In addition, there have been reports of increased infection due to bacteria with intrinsic resistance to colistin, such as *Proteus* spp., *Providencia* spp., *Serratia* spp., and *Morganella* spp ***(Samonis et al., 2014; Aghapour et al., 2019)***.

B. Activity spectrum of colistin

Colistin is a narrow-spectrum antimicrobial agent that has significant activity against most members of the *Enterobacteriaceae* family, including *Escherichia coli*, *Enterobacter* spp., *Klebsiella* spp., *Citrobacter* spp., *Salmonella* spp., *and Shigella* spp. It also has activity against common non fermentative Gram-negative bacteria, such as *Acinetobacter baumannii, Pseudomonas aeruginosa, and Stenotrophomonas maltophilia* ***(Tan and Ng, 2006; Bialvaei et al., 2017).***

In addition, *Haemophilus influenzae, Legionella pneumophila, Aeromonas* spp*., and Bordetella pertussis* are naturally susceptible to colistin ***(Giamarellou and Poulakou, 2009; Biswas et al., 2012)***.

Conversely, among the *Enterobacteriaceae, Proteus* spp. *and Serratia marcescens* have intrinsic resistance to colistin. On the other hand, *Morganella morganii, Providencia* spp., *Pseudomonas mallei, Burkholderia cepacia, Chromobacterium* spp*., Edwardsiella* spp*., Brucella and Vibrio cholera* are typically resistant to colistin. Colistin is not active against Gram negative cocci, such as *Neisseria* spp., Gram-positive bacteria, anaerobic bacteria, eukaryotic microbes, or mammalian cells ***(Gales et al., 2011; Bialvaei et al., 2015)***.

C. Mechanisms of colistin resistance in Enterobacteriaceae:

Although the main mechanism of resistance to colistin is unclear, Gram-negative bacteria employ several mechanisms to protect themselves against colistin toward other polymyxins (Figure 1). According to the literature, most colistin-resistance mechanisms are adaptive mechanisms that occur after in vitro exposure ***(Biswas et al., 2012)***.

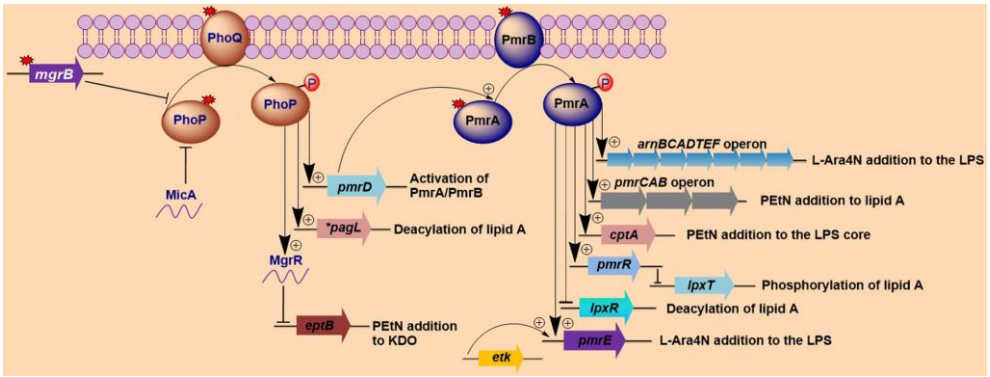

Figure (1): Activation of lipo poly saccharide-modifying genes involved in polymyxins resistance in Gram-negative bacteria ***(Biswas et al., 2012)***.

Resistance to colistin occur with LPS modification via different routes. The most common strategies for resistance to colistin are modifications of the bacterial outer membrane through alteration of the LPS and reduction in its negative charge ***(Landman et al., 2008; Nation and Li, 2009)***.

The other strategy is the over expression of efflux-pump systems**.** Also another mechanism is overproduction of capsule polysaccharide, No enzymatic mechanisms of resistance have been reported, but strains of *P. polymyxa* produce colistinase ***(Padilla et al., 2010; Kim et al., 2014)***.

1. <u>Intrinsic resistance mechanisms:</u>

Resistance to polymyxins occurs naturally in *P. mirabilis* and *S. marcesens* by modification of the LPS via cationic substitution. The mechanism of resistance in these species is linked to expression of the *arnBCADTEF* operon and the *eptB* gene and *eptC* gene. In this way, the 4-amino-4-deoxy-L-

arabinose (L-Ara4N) and phospho ethanolamine (pEtN) cationic groups are added to the LPS by this operon and those genes, respectively ***(Boll et al., 1994; Aquilini et al., 2014)***.

Putative loci in *P. mirabilis* include the sap operon encoding a transport protein, ATPase gene, and O-acetyl transferase gene, which take part in biosynthesis or transfer of amino arabinose ***(McCoy et al., 2001)***.

Similarly, this operon is responsible for intrinsic resistance to colistin in *S. marcescens*, as it has been shown that *arnB* and *arnC* mutants lead to a reduction insusceptibility to colistin (minimum inhibitory concentration [MIC] from 2,048 to 2 μg/ mL) compared to the wild type ***(Lin et al., 2014)***.

This modification of LPS and the increase in its charge decrease the affinity of colistin for binding to LPS. Therefore, intrinsic resistance has occurred in these species ***(Aquilini et al., 2014; Poirel et al., 2017)***.

2. Acquired resistance mechanisms in *Enterobacteriaceae*:

Acquired colistin-resistance mechanisms have been recognized in some members of *Enterobacteriaceae* family, such as *E. coli*, *Salmonella* spp., *Klebsiella* spp., *and Enterobacter* spp., and remain unknown for other bacterial species. Resistance mechanisms are presumed to be linked to chromosomal mutation untransferable via horizontal gene transfer ***(Lee et al., 2016)***.

Only one mechanism of resistance has been identified as a transferable mechanism (plasmid-mediated mcr gene) ***(Poirel et al., 2017; Aghapour et al., 2019)***.

Many genes and operons play a role in modification of LPS, which in turn leads to colistin resistance as some of these mechanisms were shown in figure (2). These include: genes and operons responsible for encoding enzymes that have a direct role in LPS modification, such as the *pmrC* and *pmrE* genes, the *pmrHFIJKLM* operon, regulatory two-component systems (TCSs); including *PmrAB*, *PhoPQ* and *crrAB*, which regulates the *PmrAB* system and plasmid-mediated *mcr* genes. Cpx and Rcs which are regulators of upregulation of capsule biosynthesis are also activator of the efflux pump *KpnEF* and regulating the *PhoPQ* system, respectively ***(Baron et al., 2016; Cheng et al., 2016).***

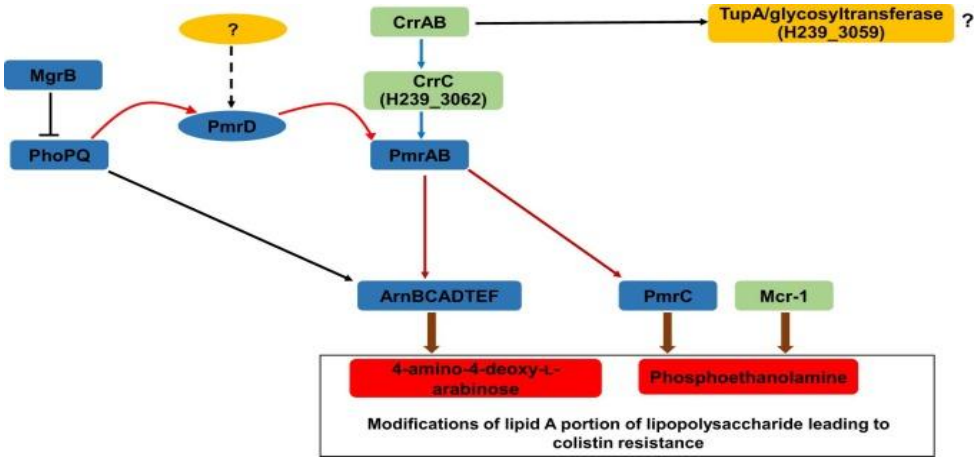

Figure (2): Model for activation of the two-component systems (TCSs) for colistin resistance in bacteria ***(Baron et al., 2016).***

a) Genes and operones that play a role in lipo polysaccride modification

i. mgrB gene and regulators of PmrAB and PhoPQ two component systems

Some operons and regulators have a role in the modification of LPS by *PmrAB* and *PhoPQ* TCSs. The *pmrABC* operon encodes *PmrA (BasR)* as a regulator protein, *PmrB (BasS)* as a cytoplasmic membrane-bound sensor kinase, and *PmrC* as a putative membrane protein ***(Gunn, 2008)***. The addition of L-arabinose amine (L-Ara4N) to the 1-phosphate or 4′-phosphate group leads to colistin resistance ***(Olaitan et al., 2014)***.

Under environmental stimulants, such as macrophage phagosomes, the high concentration of iron (Fe3+) and exposure to aluminum (Al3+), as well as acidic pH, leads to activation of *PmrB* ***(McPhee et al., 2003; Gunn, 2008)***.

Mutation within the *pmrA* and *pmrB* genes leading to colistin resistance has been described in *Klebsiella pneumoniae* and *Salmonella entericca* ***(Olaitan et al., 2015; Nordmann et al., 2016)***.

On the other hand, the *phoPQ* TCS encodes *PhoP* as a regulator protein and *PhoQ* as a sensor kinase. Under conditions of low magnesium or calcium, acidic PH, or cationic antimicrobial peptide, *PhoPQ* is activated and protects bacteria ***(Moskowitz et al., 2004; Aghapour et al., 2019)***.

Activated *PhoPQ* leads to modification of lipid A via two routes: *PhoQ* activates *PhoP* by its kinase activity via phosphorylation, which activates transcription of the *pmrFHIJKLM* operon, followed by modification of lipid A*;* and *PhoP* indirectly activates *pmrA* by bypassing the *PmrD* connector protein, subsequently activates the transcription of the *pmrHFIJKLM* operon and synthesizes PETN, which transfers it to lipid A ***(Cheng et al., 2010; Park and Groisman, 2014)***.

Mutation of the *phoP/Q* genes has been identified in *K. pneumoniae* and *E. coli* that led to acquired colistin resistance ***(Cheng et al., 2015; Nordmann et al., 2016)***.

The *mgrB* gene encodes a small transmembrane protein of 47 amino acids that exerts negative feedback on the *PhoPQ* TCS ***(Lippa and Goulian, 2009)***.

This protein inhibits the kinase activity of *PhoQ*, which in turn represses expression of the *phoQ* gene. Nevertheless, mutation/inactivation of the *mgrB* gene results in upregulation of the *phoPQ* operon and subsequent activation of the *pmrHFIJKLM* operon. Finally, production of L-Ara4N leads to modification of lipid A and colistin resistance ***(Cannatelli et al., 2013)***.

Various mutations or disruptions of the *mgrB* gene have been reported, such as deletion, nonsense, missense, inactivation, and insertional mutations. According to reports,