

Resistance to colistin in *Klebsiella pneumoniae*: a 4.0 strain?

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Abstract

The global rise of multidrug-resistant gram-negative bacteria represents an increasing threat to patient safety. From the first observation of a carbapenem-resistant gram-negative bacteria a global spread of extended-spectrum beta-lactamases and carbapenemases producing *Klebsiella pneumoniae* has been observed. Treatment options for multi-drug-resistant *K. pneumoniae* are actually limited to combination therapy with some aminoglycosides, tigecycline and to older antimicrobial agents. Unfortunately, the prevalence of colistin-resistant and tigecycline-resistant *K. pneumoniae* is increasing globally. Infection due to colistin-resistant *K. pneumoniae* represents an independent risk factor for mortality. Resistance to colistin in *K. pneumoniae* may be multifactorial, as it is mediated by chromosomal genes or plasmids. The emergence of transmissible, plasmid-mediated colistin resistance is an alarming finding. The absence of new agents effective against resistant Gram-negative pathogens means that enhanced surveillance, compliance with infection prevention procedures, and antimicrobial stewardship programs will be required to limit the spread of colistin-resistant *K. pneumoniae*.

The carbapenemases' globalization

The global rise of multidrug-resistant (MDR) gram-negative bacteria is alarming and represents an increasing threat to healthcare delivery and patient safety in Europe and beyond.¹ *Klebsiella pneumoniae* (*K. pneumoniae*) is a rod-shaped Gram-negative bacterium representing the most significant member of the *Klebsiella* genus of Enterobacteriaceae. Since the first description of resistance to β -lactam antibiotics conferred by the production of extended-spectrum-lactamases (ESBLs) in 1983,² the prevalence of ESBL-producing *K. pneumoniae* has rapidly increased. Indeed, during the past decades carbapenems were the antibiotic class of choice to face infections caused by penicillins, cephalosporins and quinolones resistant strains of *K. pneumoni-*

ae, with the (inevitable) first description of a carbapenemase – a specific carbapenem-hydrolyzing β -lactamases – in 1993,³ followed by the first observation of a carbapenem-resistant *K. pneumoniae* strain in 1999.^{4,6} From then on, a global spread of ESBLs and carbapenemases gram-negative bacteria has been observed, primarily due to *K. pneumoniae*. It is now well known that carbapenem resistance can be the result of various mechanisms including the production of a carbapenemase enzyme (metallo- β -lactamases, *K. pneumoniae* carbapenemases, OXA carbapenemases) and the combination of porins defect plus ESBL or AmpC enzyme production.⁷ Although the spread of carbapenemases appears to be quite recent, NDM, KPC, and OXA carbapenemases are now widely distributed and important reservoirs have been identified: the Indian subcontinent for KPC, NDM and OXA-181, the USA, Israel, Greece and Italy for KPC, and Turkey and North Africa for OXA-48.⁸ The reservoirs of these carbapenemase producer act as significant sources for their dissemination worldwide.⁸ As the rapid increase in the prevalence of ESBLs and carbapenem resistant gram-negative bacteria such as MDR *K. pneumoniae* is stressing the need for treatment alternatives, treatment options actually in the pipeline are limited to combination therapy with some aminoglycosides, tigecycline and to older antimicrobial agents. Polymyxins, e.g. colistin, has been reconsidered as a valid therapeutic option, in particular for critically ill patients.^{7,9}

Fighting with a naked gun

Tigecycline, an extended-spectrum glycylicycline, acts as a protein synthesis inhibitor reversibly binding the ribosome 30S subunit and interfering with accommodation of aminoacyl-tRNA in the A site of the 16S rRNA.¹⁰ In the U.S., an high rate of tigecycline resistance in *K. pneumoniae* has been recently reported in a multi-center, prospective cohort study on 287 hospitalized patients.¹¹ In this study, Van Duin *et al.* found that 46% of the cases with carbapenem resistant *K. pneumoniae* were also tigecycline-intermediate or -resistant.¹¹ In literature, among the variables described as associated with tigecycline resistance there are admission from a nursing home setting and previous antibiotic exposure,¹¹ including exposure to tigecycline and antibiotics other than tigecycline.^{11,12} In general terms, it seems that increased antimicrobial resistance to tigecycline in *K. pneumoniae* is not associated with decreased virulence and fit-

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ness, and tigecycline resistance in *K. pneumoniae* bacteremia was found associated with higher mortality rate.¹³ The mechanisms underlying tigecycline resistance are not completely understood. Previous studies attributed tigecycline resistance in Enterobacteriaceae to the increased expression of resistance-nodulation-cell division (RND) type efflux pumps, causing the reduction of the intracellular concentration of tigecycline.^{10,14,15} The *RamA*-AcrAB and *RarA*-OqxAB efflux pumps pathways are involved in tigecycline resistance in *K. pneumoniae*.¹⁰ The upregulation of transcriptional activators (such as *RamA*, *MarA*, *RarA*, and *SoxS*) can lead to increased expression of the AcrAB or the OqxAB efflux systems, having a key role in the development of tigecycline resistance. Lin *et al.* found various mutations in the negative regulatory genes *ramR* and *oxqR* determining overexpression of *ramA* and *rara*, respectively,¹² while Fang *et al.* adopted the construction of a *lon* gene mutant *K. pneumoniae* to confirm the association of mutations in the *lon* gene, an efflux-pump regulator, with tigecycline resistance.¹⁶ Recently, Villa *et al.* applied a genome sequencing approach to identify genetic mutations associated with resistance to tigecycline in *K. pneumoniae*.¹⁵ In this study, the authors identify a potential novel resistance mechanism in the mutation of the *rpsJ* gene. *RpsJ* is a gene coding for riboso-

mal protein S10-30S, reported to mediate tigecycline resistance in *K. pneumoniae* by altering the ribosome structure near the tigecycline-binding site, leading to weaker binding of tigecycline to the 16S rRNA.¹⁵ Finally, in 2014 Nielsen *et al.* observed the *in vivo* development of another mechanism of tigecycline resistance, the *kpgABC* gene overexpression due to an IS5 insertion element.¹⁷ Collectively, these results show the emergence of *K. pneumoniae* strains combining both virulence and multidrug resistance features, a matter of future concern.^{12,13}

Last resort drugs

Colistin (polymyxin E) is a cyclic polypeptide bactericidal agent possessing targeted Gram-negative activity.⁹ The polymyxins are surface active amphipathic agents acting in a detergent-like fashion to disrupt the structure of the cell membrane. The electrostatic interaction between the cationic polypeptide antimicrobial and the anionic lipopolysaccharides of the bacteria outer membrane leads to a leakage of cellular contents and, ultimately, bacterial cell lysis and killing.¹⁸ Another antibacterial mechanism of the polymyxins has been described, consisting in the inhibition of type II NADH-quinone oxidoreductases (NDH-2), vital respiratory enzymes, in the bacterial inner membrane.^{19,20} Colistin had long been kept as a reserve agent because of safety concerns, in particular regard to nephrotoxicity and neurotoxicity. The exact mechanisms of these adverse events are not well understood and despite the use of newer preparations, it is still substantial with neurotoxicity and nephrotoxicity reported in 4-6% and 14-53% of patients, respectively.^{18,21-24}

Don't be late for school again, boy

Unfortunately, with the current increase in the use of colistin for the treatment of MDR Gram-negative bacterial infections, the presence of colistin-resistant gram-negative pathogens has been reported worldwide, with a number of reports of outbreaks of Enterobacteriaceae displaying resistance to colistin.^{25,26} On a survey on 344 isolates of KPC-producing Enterobacteriaceae sent to the Centers for Disease Prevention and Control (CDC) for evaluation from January 2007 through October 2009, 9% of the isolates were resistant to colistin.²⁷ A large study collecting isolates from 2011-2012 from 31 European medical centres demon-

strated significant resistance levels in *K. pneumoniae*,²⁸ and in the EARS-Net report from 2013, 8.8% of the *K. pneumoniae* isolates were colistin-resistant, and a majority of the isolates were from Greece, Italy, Romania and Hungary; of concern, 32% of carbapenem-resistant isolates were resistant to polymyxins.^{29,30} The prevalence of colistin-resistant *K. pneumoniae* is increasing globally, with such infections becoming a critical threat to human health. As reported by Capone *et al.*, infections due to colistin-resistant *K. pneumoniae* have a higher mortality rate and colistin-resistant *K. pneumoniae* infections represent an independent risk factor for mortality.³¹

A 4.0 resistant pathogen

It is well known that resistance to polymyxins in *K. pneumoniae* can be mediated by chromosomal genes (with the most common mutations in *mgrB*, *phoP/phoQ*, *pmrA*, *pmrB*, *pmrC*, and *crrABC*).^{20,32} Resistance commonly occurs through mutations in the regulatory system PhoPQ-PmrAB, which leads to upregulation of the *pbgPE* operon and to the addition of 4-amino-4-deoxy-L-arabinose to the bacterial lipopolysaccharide (LPS) structure. This modification alters the charge of the bacterial outer membrane, reducing its interaction with colistin.³³⁻³⁵ The wide span of mutations includes silent mutations, point mutations, insertions or deletions. Therefore, resistance to colistin may be multifactorial. Of note, many chromosomally encoded mechanisms of resistance remain to be identified, and it is difficult to extrapolate whether some substitutions identified in proteins known to be involved in LPS biosynthesis lead to resistance or not. Also, the levels of expression of the corresponding genes may vary and consequently influence the level of resistance to polymyxins.²⁰ More recently, plasmid mediated colistin resistance mechanism have been reported,^{36,37} i.e. *mcr-1* and *mcr-2*, and interestingly, Cannatelli *et al.* have identified a new genetic mechanism responsible for the emergence of colistin resistance, demonstrating that *IS5-like* elements found on several *Klebsiella* KPC-encoding plasmids can cause insertional inactivation of the *mgrB* gene.³⁸ *MgrB* exerts a feedback on the PhoQ/PhoP signaling system.³⁴ Therefore, it seems that the acquisition of these plasmids could facilitate the emergence of colistin resistance.³⁸

The emergence of transmissible, plasmid-mediated colistin resistance is an alarming finding. These mechanism of transmissible resistance may accelerate the

progression of multi-drug resistant Enterobacteriaceae to pan-drug resistant Enterobacteriaceae and may ultimately cause a global spread of pan-drug resistance.^{36,39} Interestingly, as recently discussed by Poirel *et al.*, not only the novel finding of these plasmid mediated mechanisms of resistance represent cause of alarm, but it also may represent an opportunity for the adoption of molecular diagnostic techniques in the field of antimicrobial resistance detection. In fact, as the chromosomally encoded mechanisms of polymyxin resistance can be identified by sequencing specific genes, molecular techniques cannot be certainly envisioned in these case, because of their identification detects only potential resistance and is still inferior to phenotypic methods of polymyxin resistance detection. Conversely, the molecular identification of plasmid-borne *mcr-1/mcr-2* genes seems reasonable, since identification of the genes may be considered a signature of resistance and the qualitative genetic results may be translated directly into a nonsusceptibility phenotype.²⁰ According to Poirel *et al.*, in the near future screening techniques for *mcr-1/mcr-2* genes detection may be therefore applied to extensive surveillance studies.²⁰

Newer version, newer threat!

The spread of colistin resistance is a serious cause for concern, particularly in countries with high rates of carbapenem resistant Enterobacteriaceae. Management of colistin resistant *K. pneumoniae* infections presents major challenges and the increasing number of resistance mechanisms reported in such a short time period is alarming. The early identification of colistin resistant strains, at least in hospital settings, may contribute to limiting their spread.⁴⁰ Indeed, among the risk factors found associated with the occurrence of colistin resistance there is prior colistin use and duration, that could relate to the *in vivo* emergence of colistin resistance after its use, but also factors related to hospitalization such as a history of prior surgical interventions and intensive care unit stay.⁴⁰ If inappropriate usage of colistin in the clinical setting should be avoided, as selection pressure may contribute to colistin resistance due to mutations, the absence of new agents effective against resistant Gram-negative pathogens means that enhanced surveillance, compliance with infection prevention procedures, and antimicrobial stewardship programs will be required to limit the selection and patient-to-patient transmission of the threatening 4.0 colistin-resistant *K. pneumoniae*.

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