BRIEF REPORT

COLISTIN RESISTANCE IN MULTIDRUG-RESISTANT *Klebsiella pneumoniae* STRAINS AT A PERINATAL MATERNAL INSTITUTE IN LIMA, PERU, 2015-2018

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ABSTRACT

The objective of this study was to evaluate the presence of colistin- and carbapenemic-resistant genes in multidrug-resistant *Klebsiella pneumoniae* strains isolated at the Instituto Materno Perinatal de Lima (2015-2018). Susceptibility levels were analyzed by disk diffusion and microdilution. The presence of colistin- and carbapenemic-resistant genes was determined by polymerase chain reaction (PCR) and was related to clonality. A total of 36 *K. pneumoniae* strains were analyzed, 5 (13.8%) were resistant to colistin and belonged to different clonal groups. Only 2 strains were found with carbapenemases (bla_{KPC} and bla_{NDM}), and no colistin plasmid genes were detected. High resistance levels to the other tested antimicrobials were observed, except for amikacin (13.9%). The results highlight the presence of colistin-resistant strains (33.3% in 2018), a worrying situation as they are part of the latest treatment alternatives for infections caused by multiresistant pathogens.

Keywords: *Klebsiella pneumoniae*; Antimicrobial Drug Resistance; Colistin; beta-Lactams (Source: MeSH NLM).

INTRODUCTION

Antimicrobial resistance continues to be a major global health problem, mainly in developing countries [1]. Latin America is no stranger to this problem, where the growing spread of resistant bacteria threatens to increase treatment failures, even while using top-of-the-line antimicrobials [2,3]. The number of multidrug resistant bacteria associated with infectious diseases in humans increases constantly. The World Health Organization (WHO) highlights *Klebsiella pneumoniae* as one of the most important multidrug resistant bacteria for research [1], due to its ability to accumulate and disseminate antimicrobial resistance genes, and to its high plasmid load and wide variability in guanine + cytosine (G+C) content [4]. Due to the decrease in the number of effective antimicrobials for infections caused by multidrug resistant microorganisms, polymyxins (polymyxin B and polymyxin E, also called colistin) are considered as last-resort treatments, especially in severe infections by carbapenem-resistant *K. pneumoniae*. Colistin is often used in combination with other antibiotics such as tigecycline, meropenem, gentamicin, or fosfomycin to improve its spectrum and effectiveness [5].

However, in recent years, reports of *K. pneumoniae* with low sensitivity to colistin are increasing worldwide [6]. Resistance mechanisms occur due to modifications in the integrity of the cell membrane and affect bacterium anchorage, either at the lipopolysaccharide level

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or by ionic alteration of membrane (by changes in Mg+ and Ca++) [7]. These modifications can be due to mutations in chromosomal genes [6,7] and to mobile resistance genes transmissible via plasmids (*mcr*) [8].

Even though in 2016 the Pan American Health Organization (PAHO) warned of the increasing prevalence of colistin resistance (based on the first reports from 2015 of mcr-1 gene presence and the importance of its surveillance) [8], little has been studied on the evaluation of molecular mechanisms prevalence of this type of resistance and its clinical relevance in Peru.

The aim of this study is to evaluate the levels and mechanisms of resistance to colistin and carbapenems in strains of multidrug resistant *K. pneumoniae* isolated during 2015 2018 at the Instituto Nacional Materno Perinatal de Lima.

KEY MESSAGES

Motivation for the study: Colistin is one of the last-resort antibiotics to treat infections caused by multi-drug resistant bacteria. Epidemiological studies of colistin resistance in Peru are scarce.

Main findings: We analyzed multidrug resistant strains of Klebsiella pneumoniae (2015-2018). The five colistin-resistant strains were collected in 2018 and different clonal groups were found, indicating, possibly, that colistin-resistance is due to the widespread of use of this antimicrobial.

Implications: The presence of colistin-resistant strains in multidrug-resistant strains makes it difficult to choose a treatment. It highlights the need for colistin resistance surveillance systems in healthcare settings.

THE STUDY

This is a descriptive cross-sectional study of *K. pneumoniae* isolates from biological samples, such as serum, cerebrospinal fluid, bronchial aspirate, and stool culture, obtained from patients admitted to the Instituto Nacional Materno Perinatal de Lima.

We included 36 *K. pneumoniae* isolates from different patients in the study. Isolates were selected from a collection of bacteria from the Microbiology Laboratory of Instituto Nacional Materno Perinatal de Lima. Only those with multidrug resistance (MDR) sensitivity profile were considered, i.e. resistant to at least three different families of antimicrobials, as determined by disk diffusion tests. These isolates were collected between 2015 and 2018 [9].

Antimicrobial sensitivity tests were performed using disk diffusion tests. This resistance type was interpreted according to Clinical and Laboratory Standard Institute (CLSI) guidance [10]. Colistin resistance assessment was carried out by microdilution to determine the minimum inhibitory concentration (MIC) as recommended by the joint group CLSI- European Committee on Antimicrobial Susceptibility Testing (EUCAST) on polymyxin cut points [11].

We made a 1/1000 dilution in Mueller Hinton II broth (MHII) from an inoculum at 0.5 McFarland. Initially, we prepared an antibiotic solution concentration four times the concentration needed, because the bacterial inoculum (50 µL) and 50 µL of MHII medium, when added, decrease the concentration to the desired level for the serial dilutions. Each sample included a growth control well (without antibiotic) and a negative control well (MHII without the bacterial inoculum). All strains were stored at –70 °C until testing. A MIC of at least 4 mg/mL was used as a cut-off point to determine resistant strains. Escherichia coli ATCC 25922 and *Serratia* spp. were used as controls.

A MDR microorganism was defined as a microorganism not susceptible to at least one antimicrobial agent from three unrelated antimicrobial groups.

Clonality between *K. pneumoniae* isolates was defined according to the previously reported technique of pulsed field gel electrophoresis (PFGE) [9]. We considered that strains with an identity level of 100% were from the same clone, and those with an identity higher than 80% were clonally related [12].

We confirmed the presence of carbapenemase-coding genes by the multiple polymerase chain reaction test (PCR Multiplex) for blaNDM, blaVIM, blaIMP, blaKPC, and blaOXA-48 genes [13] in samples with low sensitivity to carbapenems. We carried out a PCR test to identify mcr-1 to mcr-5 genes, which are related to plasmid colistin resistance [14].

The Institutional Scientific Committee of Universidad Científica del Sur and the Scientific Committee of Instituto Nacional Materno Perinatal reviewed and approved this study.

FINDINGS

Characteristics of the population

Thirty-six isolates of MDR *K. pneumoniae* were included in our study. According to the origin of the sample, 23 strains (63.9%) were isolated from blood cultures; 9 (25%) from secretions; and 4 (11.1%) from stool cultures.

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Antimicrobial sensitivity

We found that 97% of the isolates had low sensitivity to cotrimoxazole. Regarding beta lactams, we detected 26 (72.2%) isolates with low sensitivity to aztreonam, 23 (63.8%) to ceftazidime and 32 (88.8%) to cefotaxime. Likewise, 24 isolates (66.6%) presented high resistance to gentamicin and 20 (55.5%) to amoxicillin/clavulanic acid. The level of resistance to quinolones was 36% and 47% for levofloxacin and norfloxacin, respectively. Resistance levels were close to 25% for imipenem, chloramphenicol, piperacillin-tazobactam and nitrofurantoin. It should be noted that only for amikacin, the value obtained was less than 15%.

Extended spectrum beta-lactamases (ESBL) were found in 78% of the strains. Of the isolates studied, 5 (13.8%) were resistant to colistin during the period 2015-2018. The frequency of resistant strains from 2018 was 33.3%. The mentioned resistant strains presented a MIC higher than 2 mg/L, in a range from 8 mg/L to 64 mg/L and were isolated from blood cultures (3/5) and secretions (2/5).

Molecular analysis of resistance to carbapenems and colistin

Two genes (bla_{KPC} *and* bla_{NDM}) were detected in the strains with low sensitivity to carbapenems. The strains were isolated from stool culture (code K80), and secretions (K81), respectively, both from 2018 (Figure 1).

Regarding the colistin resistant strains, PCR did not show the presence of any *mcr* gene.

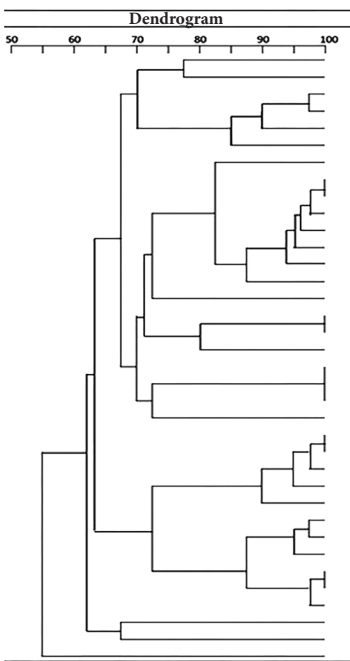
Clonality analysis

The analyzed strains belonged to 16 different pulse types that have been previously reported [14]. It is important to highlight that the strains showing levels of colistin resistance belonged to four different clones according to PFGE analysis and that all of them were found in 2018 (Figure 1).

DISCUSSION

This study demonstrates the presence of multidrug resistant strains of *K. pneumoniae* that are resistant to colistin, isolated from patients of Instituto Materno Perinatal during 2018. We found that this is not due to plasmid genes (*mcr*), at least not from *mcr1-5*.

Overall, antimicrobial resistance rates among common pathogens in Lima hospitals are high [15]. Hospital outbreaks of ESBL-producing *Klebsiella*, as well as enterobacteria, have been steadily increasing in Latin America. High incidence of ESBL (50%) has been recently described in bacteremia isolates from hospitalized patients in Peru. And the data for community-acquired infections is similar, incidence is close to 40% [15], which suggests, according to the reported findings, that presence of ESBL in the population is high [15]. It should be



ID	Pulse type	Year	Origin	ESBL	Colistin	Ab Resistence
K81	1	2018	Secretions	+	-	Mer,Imi,Ptz,Chl,Sxt,Nit,Lev,Azm,Cip,Ctx,Caz,Gen,Amc
K23	2	2015	Blood culture	-	-	Mer,Imi,Ptz,Chl,Sxt,Nit,Azm,Ctx,Caz,Gen,Amc
K28	3	2017	Blood culture	+	-	Sxt,Azm,Ctx,Caz,Gen,Amc
K64	3	2015	Blood culture	+	-	Sxt,Nit,Lev,Azm,Cip,Ctx,Caz,Gen,Amk,Amc
K1	3	2017	Blood culture	+	-	Imi,Ptz,Sxt,Nit,Azm,Ctx,Caz,Gen,Amk,Amc
K10	3	2017	Blood culture	+	-	Sxt,Lev,Azm,Cip,Ctx,Caz,Gen,Amk,Amc
K68	6	2018	Stool culture	+	-	Sxt,Ctx,Caz,Amc
K6	7	2015	Blood culture	+	-	Sxt,Azm,Ctx,Caz,Gen
K16	7	2015	Secretions	+	-	Sxt,Azm,Cip,Ctx,Caz,Gen,Amc
K2	7	2016	Blood culture	+	-	Mer,Imi,Sxt,Lev,Azm,Cip,Ctx,Caz,Gen,Amk
K30	7	2015	Blood culture	+	-	Sxt,Azm,Ctx,Gen
K11	7	2015	Blood culture	+	-	Imi,Ptz,Chl,Sxt,Nit,Ctx,Gen,Amc
K22	7	2015	Blood culture	+	-	Sxt,Azm,Cip,Ctx,Caz,Gen
K20	7	2018	Blood culture	+	64ug/ml	Sxt,Lev,Azm,Ctx,Caz,Gen,Amc
K60	8	2015	Stool culture	-	-	
K66	9	2018	Secretions	+	-	Chl,Sxt,Lev,Cip,Ctx,Caz,Gen
K82	9	2018	Secretions	-	-	Ptz,Chl,Sxt,Nit,Gen
K47	10	2018	Blood culture	-	-	Sxt,Azm,Ctx,Gen
K83	12	2018	Blood culture	+	32ug/ml	Imi,Sxt,Nit,Azm,Cip,Ctx,Caz,Gen,Amc
K79	12	2018	Secretions	-	32ug/ml	Imi,Ptz,Chl,Sxt,Nit,Lev,Azm,Cip,Ctx,Caz,Gen,Amc
K80	12	2018	Stool culture	+	-	Imi,Ptz,Chl,Sxt,Azm,Ctx,Caz,Amk,Amc
K27	13	2015	Blood culture	+	-	Sxt,Azm,Ctx,Caz,Amc
K42	14	2018	Secretions	+	-	Ptz,Sxt,Azm,Cip,Ctx,Caz
K61	14	2018	Blood culture	+	-	Sxt,Ctx,Caz,Amc
K12	14	2018	Blood culture	+	-	Imi,Chl,Sxt,Ctx,Amc
K33	14	2018	Secretions	+	-	Sxt,Azm,Cip Ctx,Caz,Amc
K29	15	2018	Blood culture	-	-	Sxt,Azm,Ctx
K8	15	2015	Stool culture	+	-	Sxt,Lev,Azm,Cip,Ctx,Caz,Gen,Amc
K51	16	2015	Secretions	+	-	Sxt,Ctx,Amc
K50	16	2015	Blood culture	+	-	Sxt,Lev,Azm,Cip,Ctx,Caz,Gen,Amc
K17	16	2015	Blood culture	+	-	Sxt,Lev,Azm,Cip,Ctx,Caz,Gen,Amc
K26	16	2018	Blood culture	+	8ug/ml	Sxt,Lev,Azm,Cip,Ctx,Caz,Gen
K4	16	2015	Blood culture	+	-	Imi,Sxt,,Lev,Azm,Cip,Ctx,Caz,Gen,Amk,Amc
K15	17	2015	Blood culture	-	-	Imi,Sxt
K67	18	2018	Secretions	-	16ug/ml	Sxt
K14	19	2015	Blood culture	+	-	Ptz,Nit,Azm,Cip,Ctx,Caz,Gen

Figure 1. Dendrogram of the clonal relation and antimicrobial sensitivity profile of the *Klebsiella pneumoniae* strains analyzed in this study.

noted that among the different pathogens reported, *Klebsiella* spp. leads with reported rates of up to 75% of resistance to third generation cephalosporins in the region [16].

Resistance to carbapenems is increasing in Peru, consistent with the growing trend reported worldwide [17-18]. We found the *bla*KPC gene in several strains of this study, which was reported for the first time in Peru in 2013 in a *Klebsiella* strain [17]. We also identified an isolate with the blaNDM gene, which is increasingly reported in Peru [18].

No colistin-resistant strains have been reported in the period 2015-2017, but in 2018, 5 isolates were found and were classified in 4 unrelated clones/pulse types. These results are aligned with the increased use of this antimicrobial in the hospital, and alert that resistant strains are already circulating in the institution. Previous studies in Peru report a level of 5% of resistance to this antimicrobial [7], and outbreaks of bla_{KPC}-producing and colistin-resistant *Klebsiella* strains have already been reported in the region [19].

Generally, resistance to colistin correlates with decreased affinity of polymyxin antibiotics for the lipid A of lipopolysaccharides. In this study, no plasmid-associated genes for colistin resistance were found, at least those tested for mcr-1 through 5. This suggests that colistin resistance is due to chromosomal mutations associated with changes in the two components (*pmrAB* and *phoPQ*) and their regulator *mgrB* [20]. In a Peruvian study from 2018, the mcr-1 gene was detected in *E. coli* strains from urine cultures, but with low frequency [20]; however, we suggested to monitor plasmid mechanisms for their ability to spread between different bacterial species. Currently, not all public hospitals monitor colistin resistance, so it seems likely that levels of resistance in Peru are underestimated.

Although this study contains a reduced sample size from a single institution, its importance lies in describing the presence of colistin-resistant strains, not by transferable mechanisms, but by widespread antimicrobial use as suggested by the clonality study. Recently, the Peruvian Ministry of Agriculture and Irrigation has prohibited the use, import, marketing, manufacture, or processing of veterinary products containing colistin (polymyxin E) to avoid the selective pressure of this antimicrobial, because it is a top priority for medical use.

In conclusion, we found a high percentage of colistin-resistant strains, not clonally related. It is important to note that this antimicrobial is part of the last-resort treatment alternatives. Therefore, it is urgent to establish screening systems and epidemiological surveillance of colistin resistance in all hospitals in the country.

Authorship contributions: GS and MJP conceived the study. GS collected the data at the hospital. BY, ANM, MV and MJP performed the experiments and data analysis. ANM, MV, BY and MJP wrote the manuscript. All the authors approved the final version of the article.

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