

Increase resistance towards colistin in carbapenem-resistant *Enterobacteriaceae* strains in a tertiary hospital, Quito, Ecuador

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Summary

The aim of the current study was to present the increased resistance in carbapenem-resistant *Enterobacteriaceae* strains towards colistin. An observational, descriptive study, based on the analysis of microbiological reports of carbapenemase-producing strains from 2015 to June 2018 in the Microbiology Department of the Hospital de Especialidades de las Fuerzas Armadas N°1 in Quito, Ecuador, was carried out.

Between 2015 and June 2018, carbapenemase-producing strains infected 67 patients, in total. Among them, males (57.89% to 81.82%), and people older than 60 years (57.33% to 90.91%), were mostly affected by the infection. Further, the most frequent pathogen isolated was *Klebsiella pneumoniae* (57.69% to 93.33%), followed by *K. oxytoca*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter freundii*, and *Serratia marcescens*. However, a reduction in the number of patients affected through the years, 24 in 2015, 19 in 2016, 13 in 2017, and 11 in 2018,

was witnessed. In all the isolates, carbapenemase *bla*_{KPC}-type producing strains with 100 % resistance towards aminopenicillins alone or combined with beta-lactamase inhibitors, ureidopenicillin, cephalosporins, ciprofloxacin, and carbapenems, were discovered. On the contrary, aminoglycosides and colistin exhibited variable resistance patterns.

In the current investigation, emerging resistance to colistin in *K. pneumoniae* in 2017 and 2018 was observed, upon being compared with the year 2015 and 2016. The observations, in the current study, are similar to other reports that show the emerging resistance to colistin. However, more studies are required to determine the impact of this problem in public health.



Key words

Carbapenem-Resistant *Enterobacteriaceae*; Colistin; Resistance

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Introduction

Carbapenems are antimicrobial agents that possess the broadest spectrum of activity and greatest potency against Gram-positive and Gram-negative bacteria. However, the increasing resistance to carbapenems has concerned all the medical community.¹ Among these strains, *Klebsiella pneumoniae* carbapenemase (KPC)-producing bacteria are one of the most frequent microbiological agents that affect people worldwide.² In this context, a metanalysis carried out in seven studies, demonstrated that the rate of deaths attributable to Carbapenem-Resistant *Enterobacteriaceae* (CRE) varies from 26 % to 44 %. Moreover, an unadjusted number of fatalities in patients affected by CRE was found to be 2-fold higher than for the patients infected by Carbapenem-Sensible *Enterobacteriaceae*.³

Colistin, which is a cyclic lipopeptide and belongs to the group of polymyxins, is an option to treat infection caused by CRE.⁴ There are five types of polymyxins out of which only two, namely (i) polymyxin B, and (ii) polymyxin E (colistin), are used in the clinical practice. However, there is evidence of increased resi-

stance to colistin by alteration of the lipopolysaccharide (LPS) moiety through the attachment of positively charged L-Ara4-N and PEtN molecules. Further, the most frequent mutations associated with the emerging resistance, are located in *mgrB*, *phoP/phoQ*, *pmrA*, *pmrB*, *pmrC*, and *crrABC*.^{5,6,7} These reports are particularly concerning because inappropriate use of colistin has been reported, leaving behind with fewer antibiotics as a last resort to treat patients infected by CRE. Therefore, this study aims to explain the core cause of the increase in resistance towards colistin in carbapenemase-producing strains isolated in the Microbiology Department (MD) of the Hospital de Especialidades de las Fuerzas Armadas N°1 (HE1) in Quito, Ecuador.

Materials and Methods

The current observational and descriptive study was carried out in July 2018, in Quito, Ecuador, based on the analysis of the microbiological reports, which mentions isolation of CRE strains from 2015 to June 2018, in the MD of HE1.

Detection of Carbapenem-Resistant Enterobacteriaceae (CRE)

Initially, the identification of CRE was carried out by using both (i) the Modified Hodge Test (MHT), and (ii) carbapenem inactivation method. The Modified Hodge Test (MHT) is based on the inactivation of a carbapenem by CRE, allows it to grow a sensible strain (*E. coli* ATCC® 25922). Alternatively, the carbapenem inactivation method, based on the use of a disc of Meropenem uses a Muller-Hinton agar (MHA) with the CRE in 400 µl of water. The disc was initially placed in this agar, followed by incubation for 24 hours at 35°C. Subsequently, the disc was transferred on a Mueller-Hinton agar plate inoculated with a susceptible *E. coli* indicator strain (*E. coli* ATCC® 25922). Additionally, a double-disk diffusion test was also employed for establishing the resistance against meropenem, imipenem, and ertapenem, as well as, RAPIDEC® CARBA NP.

Detection of acquired resistance to colistin in Enterobacteriaceae

Firstly, an automated VITEK 2 system for bacterial identification was employed, which uses a card with colorimetric reagents where pure microorganisms undergo inoculation with the susceptibility profile being interpreted automatically. Further, the analysis of obtained Colistin-resistant *Enterobacteriaceae* using the above method leads to the lack of sensitivity. However, high specificity was found, for the detection of colistin resistance. Therefore, for categorizing the isolation as sensitive, a subsequent confirmation was carried out using Rapid Polymyxin™ NP, and dilution methods.

The Rapid Polymyxin™ NP test, based on the detection of the glucose metabolism related to bacterial growth in the presence of a defined concentration of colistin, indicated a color change (orange to yellow) on a pH indicator.

The technique of dilution in agar or broth is used to measure the antimicrobial capacity “*in vitro*” of an antibiotic against a bacterium. This technique is based on the use of several tubes or plates with broth or agar to which the antibiotic was added in different concentrations, followed by the addition of equal known levels of microorganisms and overnight incubation at 37°C, after which the Minimum Inhibitory Concentration (MIC) of the antibiotic against the microorganism, was determined.

The stock solution, of the microorganism under examination, was prepared with ten times higher concentration than the last (highest) concentration to be tested. For example, if 125 µg/ml is the highest level of the colistin to be tested, then one requires preparing the stock solution with 1250 µg/ml concentration. Further, the inoculation of the antibiotic in each

solution was carried out in a liquid culture medium, where the most recommended is the Muller-Hinton broth, followed by the inoculation of the known concentration of bacteria in each of the tubes which were subsequently read.

Confirmation of our results by the National Institute of Public Health Research (Instituto Nacional de Investigación en Salud Pública, INSPI)

The detection of CRE was reported, compulsory, to INSPI, where all our findings were confirmed. They use the diffusion disc method to test the susceptibility, thereby interpreting the results according to the standards established by CLSI. Additionally, molecular biology was used, for the detection of carbapenemase *bla_{KPC}* type producing strains through PCR.

Statistical analysis

SPSS software and Excel, both in their latest versions for Windows 10, were employed for tabulation and analysis of the data.

Results

Between 2015 and June 2018, 88 CRE were identified isolates from 67 patients ; In 2015 a total of 598 *E. coli* and 226 *K. pneumoniae* were isolated, from them, 2 *E. coli* and 28 *K. pneumoniae* were CRE, During 2016 there were 572 isolates of *E. coli*, 175 *K. pneumoniae*, 21 *K. oxytoca*, 22 *S. marcescens*, and 60 *E. cloacae*, with a total de CRE in each group of 2, 15, 1, 7, 1, respectively, In 2017 a total of 540 *E. coli*, 129 *K. pneumoniae*, and 13 *C. freundii* were isolated, from them, 1 *E. coli*, 16 *K. pneumoniae*, and 1 *C. freundii* were CRE, finally from the period included of 2018 there were 105 isolates of *K. pneumoniae* and 6 of *C. freundii* with 12 *K. pneumoniae* and 2 *C. freundii* being CRE. Among the 67 patients, we found males more affected by CRE (57.89% to 81.82%), also, people older than 60 years (57.33% to 90.91%) were mostly affected. Further, the pathogen, *K. pneumoniae* (57.69% to 93.33%), was isolated most frequently. The general findings obtained in the present study are tabulated (see Table 1). In all the isolates, *bla_{KPC}*- type producing strains with 100 % resistance to aminopenicillins alone or combined with beta-lactamase inhibitors, ureidopenicillin, cephalosporins, ciprofloxacin, and carbapenems, was identified. In contrast, different resistance levels to aminoglycosides and colistin, through the years, was found. Additionally, among all the isolates obtained by us, a strain resistant to colistin was obtained, while upon testing with tigecycline, all of them were found to be sensible (see Table 2).

Table 1 General findings

| | 2015 | 2016 | 2017 | 2018* |
|---------------------------------------|------------|------------|------------|------------|
| Number of patients, n (%) | 24 (100) | 19 (100) | 13 | 11 |
| Age | | | | |
| Less than 1 year, n (%) | 1 (4.17) | 0 (0) | 0 (0) | 0 (0) |
| 1 to 14 years, n (%) | 0 (0) | 0 (0) | 1 (7.69) | 0 (0) |
| 15 to 20 years, n (%) | 0 (0) | 0 (0) | 1 (7.69) | 0 (0) |
| 21 to 40 years, n (%) | 4 (16.67) | 2 (10.53) | 1 (7.69) | 1 (9.09) |
| 41 to 60 years, n (%) | 5 (20.83) | 1 (5.26) | 1 (7.69) | 0 (0) |
| Older than 60 years, n (%) | 14 (58.33) | 16 (84.21) | 9 (69.23) | 10 (90.91) |
| Gender | | | | |
| Male, n (%) | 15 (62.50) | 11 (57.89) | 8 (61.54) | 9 (81.82) |
| Female, n (%) | 9 (37.50) | 8 (42.11) | 5 (38.46) | 2 (18.18) |
| Origin of the samples taken | | | | |
| Total number of samples, n (%) | 30 (100) | 26 (100) | 18 (100) | 14 (100) |
| Blood, n (%) | 4 (13.33) | 0 (0) | 3 (16.67) | 2 (14.29) |
| Urine, n (%) | 7 (23.33) | 7 (26.92) | 2 (11.11) | 3 (21.43) |
| Any type of wound or skin swab, n (%) | 2 (6.67) | 3 (11.54) | 1 (5.56) | 2 (14.28) |
| Tracheal aspirate, n (%) | 11 (36.67) | 9 (34.62) | 7 (38.89) | 4 (28.57) |
| Nasopharyngeal aspirate, n (%) | 0 (0) | 0 (0) | 1 (5.56) | 0 (0) |
| Sputum, n (%) | 5 (16.67) | 4 (15.38) | 1 (5.56) | 2 (14.29) |
| Bronchoalveolar lavage, n (%) | 0 (0) | 1 (3.85) | 2 (11.11) | 0 (0) |
| Rectal swab, n (%) | 0 (0) | 1 (3.85) | 1 (5.56) | 0 (0) |
| Catheter, n (%) | 0 (0) | 0 (0) | 0 (0) | 1 (7.14) |
| Bone tissue, n (%) | 1 (3.33) | 1 (3.85) | 0 (0) | 0 (0) |
| CRE isolated | | | | |
| <i>Escherichia coli</i> , n (%) | 2 (6.67) | 2 (7.68) | 1 (5.56) | 0 (0) |
| <i>Klebsiella pneumoniae</i> , n (%) | 28 (93.33) | 15 (57.69) | 16 (88.89) | 12 (85.71) |
| <i>Klebsiella oxytoca</i> , n (%) | 0 (0) | 1 (3.85) | 0 (0) | 0 (0) |
| <i>Serratia marcescens</i> , n (%) | 0 (0) | 7 (26.92) | 0 (0) | 0 (0) |
| <i>Enterobacter cloacae</i> , n (%) | 0 (0) | 1 (3.85) | 0 (0) | 0 (0) |
| <i>Citrobacter freundii</i> , n (%) | 0 (0) | 0 (0) | 1 (5.56) | 2 (14.29) |

Note. *Data from 2018 correspond to the period between January and June; CRE, Carbapenem-Resistant Enterobacteriaceae

Discussion

The infection caused by Gram-negative bacteria leads to frequent hospitalization. Therefore, antibiotics are employed broadly but are sometimes used irrationally thus leading to growth in the antimicrobial resistance.⁸ Additionally, during the last decade, an increasing pattern of resistance to carbapenems among *Enterobacteriaceae*⁹ was determined. Thus, only a few options remain for the treatment of the infections such as colistin and tigecycline. However, nowadays growing resistances to these “last-line” antibiotics are being

noticed. A recent systematic review, which included 13 studies about hospital outbreaks of colistin resistance in Europe, showed that the most affected countries were Italy and Greece. Additionally, the most frequent colistin-resistant pathogen identified was *K. pneumoniae*, found in 61.54% (n=8) of all the publications analyzed, followed by *Pseudomonas aeruginosa* in 30.77% (n=4) and *Acinetobacter baumannii* 7.69% (n=1). Also, the more frequent carbapenemase coding genes, were *bla_{KPC2}* and *bla_{KPC3}*.¹⁰ In Latin America outbreaks of *K. pneumoniae* resistant to colistin were published in Colombia and Argentina.¹¹ These findings

Table 2 Antimicrobial resistance found in every year from 2015, to 2018

| 2015 | AMP | SAM | CXM | TZP | IPM | MPM | ETP | AMK | CIP | CST |
|--------------------------------------|----------|----------|----------|----------|----------|----------|----------|------------|------------|----------|
| Pathogens isolated | | | | | | | | | | |
| <i>Escherichia coli</i> , n (%) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 1 (50) | 2 (100) | 0 (0) |
| <i>Klebsiella pneumoniae</i> , n (%) | 28 (100) | 28 (100) | 28 (100) | 28 (100) | 28 (100) | 28 (100) | 28 (100) | 19 (67.86) | 27 (96.43) | 0 (0) |
| 2016 | | | | | | | | | | |
| <i>Escherichia coli</i> , n (%) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 1 (50) | 0 (0) | 0 (0) |
| <i>Klebsiella pneumoniae</i> , n (%) | 15 (100) | 15 (100) | 15 (100) | 15 (100) | 15 (100) | 15 (100) | 15 (100) | 5 (33.33) | 15 (100) | 0 (0) |
| <i>Klebsiella oxytoca</i> , n (%) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 0 (0) | 0 (0) |
| <i>Serratia marcescens</i> , n (%) | 7 (100) | 7 (100) | 7 (100) | 7 (100) | 7 (100) | 7 (100) | 7 (100) | 1 (14.29) | 7 (100) | 7 (100) |
| <i>Enterobacter cloacae</i> , n (%) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 1 (100) | 0 (0) |
| 2017 | | | | | | | | | | |
| <i>Escherichia coli</i> , n (%) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 1 (100) | 0 (0) |
| <i>Klebsiella pneumoniae</i> , n (%) | 16 (100) | 16 (100) | 16 (100) | 16 (100) | 16 (100) | 16 (100) | 16 (100) | 0 (0) | 16 (100) | 6 (37.5) |
| <i>Citrobacter freundii</i> , n (%) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 1 (100) | 0 (0) | 1 (100) | 0 (0) |
| 2018* | | | | | | | | | | |
| <i>Klebsiella pneumoniae</i> , n (%) | 12 (100) | 12 (100) | 12 (100) | 12 (100) | 12 (100) | 12 (100) | 12 (100) | 4 (33.33) | 12 (100) | 6 (50) |
| <i>Citrobacter freundii</i> , n (%) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 2 (100) | 0 (0) | 2 (100) | 0 (0) |

Note. *Data from 2018 correspond to the period between January and June; AMP, Ampicillin; SAM, Ampicillin/Sulbactam; CXM, Cefuroxime; TZP, Piperacillin/Tazobactam; IPM, Imipenem; AMK, Amikacin; CIP, Ciprofloxacin; CST, Colistin

are concordant with our results, as in the current investigation the only pathogen resistant to colistin in 2017 and 2018, was *K. pneumoniae*, whereas *Serratia marcescens* was found to be in 2016. On the contrary, the identification of carbapenem-resistant *Pseudomonas* strains has not come across. Therefore, it is essential to highlight the fact that *S. marcescens* have intrinsic resistance to colistin, but *K. pneumoniae* generate resistance to polymyxins in a phenomenon dominated acquired resistance.¹²

Interestingly, a reduction in the patients affected by CRE, in our institution, was observed apparently due to the active work made by the microbiology and infectiology departments in the hospital. However, generalizing about other health institutions in Ecuador, was difficult. Additionally, a variety of isolated bacteria of CRE, which include *K. oxytoca*, *E. cloacae*, *E. coli*, *C. freundii*, and *S. marcescens*, were present (also mentioned in other reports). Also, in 2018 data from the period between January and June was only included. However, the most relevant limitation of the present study was the lack of more detailed analysis about the molecular biology in all the isolates. Nevertheless,

this study could serve as a base for new studies about the colistin resistance among *Enterobacteriaceae*.

In conclusion, in spite of the reduction in the isolates of CRE, there was increased resistance to colistin in *K. pneumoniae* in 2017 and 2018 compared with 2015 and 2016, which was in conformity with other reports that describe this emerging problem. However, it is important to contrast these findings with other hospitals and with other countries, for establishing the severity of the resistance to colistin in CPE concerning public health.

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Competing interests

All authors declare that they have no competing interests.

Consent for publication

Not applicable



Περίληψη

Αυξημένη αντοχή στην κολιστίνη καρβαπενέμη-ανθεκτικών εντεροβακτηριακών σε τριτοβάθμιο νοσοκομείο στο Quito του Εκουαδόρ

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Σκοπός της μελέτης ήταν η διερεύνηση αντοχής στην κολιστίνη καρβαπενέμη-ανθεκτικών εντεροβακτηριακών (Carbapenem-Resistant *Enterobacteriaceae*, CRE). Υλικό αποτέλεσαν στελέχη CRE τα οποία συλλέχθηκαν το διάστημα 2015 έως Ιούνιο 2018 στο Μικροβιολογικό Εργαστήριο του Hospital de Especialidades de las Fuerzas Armadas N°1 στο Quito του Εκουαδόρ. Ακολούθησε ανάλυση τόσο των επιδημιολογικών δεδομένων των ασθενών από τους οποίους προέρχονταν τα συγκεκριμένα στελέχη, όσο και των μικροβιολογικών δεδομένων τους. Σύμφωνα με τα αποτελέσματα της μελέτης, απομονώθηκαν 88 CRE στελέχη από 67 συνολικά ασθενείς. Από αυτούς οι πλειονότητα ήταν άνδρες (από 57.89% έως 81.82%, τα διάφορα έτη) και με ηλικία άνω των 60 ετών (από 57.33% έως 90.91%, τα διάφορα έτη). Τα πιο συχνά είδη ήταν *Klebsiella pneumoniae* (από 57.69% έως 93.33%, τα διάφορα έτη), και στη συνέχεια *K. oxytoca*, *Enterobacter cloacae*, *Escherichia coli*, *Citrobacter freundii* και *Serratia marcescens*. Παρατηρήθηκε μείωση του αριθμού των ασθενών με CRE λοίμωξη κατά τη διάρκεια των ετών της μελέτης: 24 ασθενείς το 2015, 19 το 2016, 13 το 2017 και 11 το 2018. Όλα τα στελέχη έφεραν το γονίδιο *blaKPC* και είχαν 100 % αντοχή σε αμινοπενικιλίνες, μόνες και σε συνδυασμό με αναστολείς β-λακταμασών, ουρεϊδοπενικιλίνες, κεφαλοσπορίνες, σιπροφλοξασίνη και καρβαπενέμες. Αντίθετα σε αμινογλυκοσίδες και κολιστίνη παρουσίαζαν ποικίλα αποτελέσματα αντοχής. Έτσι αντοχή στην κολιστίνη καταγράφηκε σε στελέχη *K. pneumoniae* των ετών 2017 και 2018, και όχι σε αυτά των ετών 2015 και 2016. Τα ευρήματα της συγκεκριμένης μελέτης ήταν παρόμοια με αυτά άλλων μελετών, οι οποίες επίσης παρουσιάζουν τα τελευταία χρόνια αύξηση αντοχής στην κολιστίνη. Εντούτοις, χρειάζονται περισσότερες μελέτες για να δείξουν την έκταση του συγκεκριμένου προβλήματος σε σχέση και με τη δημόσια υγεία.



Λέξεις κλειδιά

Καρβαπενέμη-ανθεκτικά *Enterobacteriaceae*;
CRE; κολιστίνη; αντοχή

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