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Brief Original Article

Macrolides: a novel risk factor for carbapenemase-producing *Enterobacterales* in intensive care units

Carmen Soria-Segarra[1,2 #], Claudia Soria-Segarra[1,2,3,4 #], Angel Catagua-González[5], Marcia Apolo-Matamoros[6], Franklin Vega-Franco[1], Miguel Chung-Sang[1,6], César Narváez-Peñaloza[1], Raquel Quijano-Grunauer[7], José Gutiérrez-Fernández[2]

[1] Instituto de Investigación e Innovación en Salud Integral, Facultad de Ciencias Médicas, Universidad Católica Santiago de Guayaquil. Guayaquil, Ecuador
[2] Department of Microbiology, School of Medicine and PhD Program in Clinical Medicine and Public Health, University of Granada-ibs. Granada, España
[3] Universidad de Guayaquil. Faculttad de Ciencias Médicas. Guayaquil-Ecuador
[4] Laboratorio Clínico Sosegar. Guayaquil,Ecuador
[5] Sociedad Ecuatoriana de Estadística. Quito, Ecuador
[6] Universidad de Especialidades Espíritu Santo. Samborondón, Ecuador
[7] Hospital Abel Gilbert Pontón. Guayaquil, Ecuador

# Authors contributed equally to the manuscript.

Abstract

Introduction: Carbapenemase-producing *Enterobacterales* (CPE) have emerged as a substantial cause of morbi-mortality worldwide, with a prevalence of approximately 5% in areas with high endemicity. However, available data may not be representative of developing countries, such as Ecuador. In this study, the incidence of CPE in Ecuador and risk factors for infection/colonisation were evaluated.

Methodology: A prospective cohort study was performed from February to April 2016 in seven intensive-care units of Guayaquil, Ecuador. Samples were processed according to the Centers for Disease Control and Prevention laboratory protocol and the CHROMagar mSuper CARBA agar method. Resistance to carbapenems was defined according to Clinical and Laboratory Standards Institute breakpoints. A modified carbapenemase inactivation method was used to identify carbapenamase production phenotypically with molecular confirmation by multiplex polymerase chain reaction.

Results: In total, 640 patients were enrolled. The incidence of CPE was 36.4% (N = 233). A multivariate analysis indicated that several factors were associated with CPE acquisition, included a long intensive care unit stay (OR 1.05; 95% CI 1.03–1.08; $p < 0.01$), tracheostomy (OR 3.52; 95% CI 1.90–6.75; $p < 0.01$), hospitalisation 3 months prior to admission (OR 2.07; 95% CI 1.17–3.71; $p < 0.01$), vancomycin use (OR 3.31; 95% CI 2.02–5.18; $p < 0.01$), and macrolide use (OR 3.31; 95% CI 1.43–7.76; $p < 0.01$).

Conclusions: Macrolide use was a risk factor for CPE acquisition. This association should be evaluated further, especially in developing countries.

Key words: Carbapenemase; Enterobacterales; risk factors; intensive care unit.

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Introduction

Carbapenemase-producing *Enterobacterales* (CPE) have emerged as a substantial cause of morbi-mortality worldwide [1], with a prevalence of approximately 5% in high endemicity areas. However, these statistics may not be representative of developing countries, such as Ecuador, where CPE has shown rapid dissemination since 2010 [2-4]. Thus, the aims of this study were to determine the incidence of CPE colonisation/infection and related risk factors in patients in intensive-care units (ICUs) in Guayaquil, Ecuador.

Methodology

Study design

A prospective cohort study was performed between February and April 2016 in seven ICUs in Guayaquil, Ecuador. All admitted patients were screened with inguinal/perineal swabs weekly and upon admission if they were transferred from other institutions or had known risk factors for CPE. Patients were excluded if they were infected or colonised with CPE at enrolment [5].

Table 1. Clinical characteristics and univariate analysis of risk factors for carbapenemase-producing *Enterobacterales.*

Characteristic	CPE (n = 233)	Non-CPE (n = 407)	RR (95% CI)	*p*-value
Female	90 (33.7%)	177 (66.3%)		0.23
Age (± IQR)*	56.00 ± 29.50	56.00 ± 29.50		0.04
APACHE II score (± IQR)*	16.00 ± 12.00	16.00 ± 12.00		< 0.01
Transfer	108 (47.2%)	121 (52.8%)	1.55 (1.27–1.89)	< 0.01
ICU stay (± IQR) (days)	21.00 ± 22.00	9.00 ± 9.00		< 0.01
Hospital stay (± IQR) (days)	27.00 ± 23.50	16.00 ± 15.00		< 0.01
Mortality	83 (44.1%)	105 (55.9%)	1.33 (1.08–1.64)	< 0.01
Invasive procedures				
Mechanical ventilation	192 (82.76%)	246 (60.44%)	2.20 (1.64–2.96)	< 0.01
Central venous catheter	211 (90.56%)	317 (77.89%)	2.03 (1.38–3.00)	< 0.01
Urinary catheter	224 (96.14%)	363 (89.19%)	2.25 (1.23–4.11)	< 0.01
Gastrostomy	25 (10.73%)	19 (4.67%)	1.63 (1.23–2.15)	< 0.01
Tracheostomy	97 (41.63%)	51 (12.53%)	2.37 (1.97–2.85)	< 0.01
Nasogastric tube	190 (81.55%)	265 (65.11%)	1.8 (1.35–2.39)	< 0.01
Haemodialysis catheter	34 (14.59%)	30 (7.39%)	1.54 (1.19–1.98)	< 0.01
Parenteral nutrition	89 (38.2%)	101 (24.82%)	1.46 (1.19–1.79)	< 0.01
Surgery	135 (57.94%)	176 (43.24%)	1.45 (1.18–1.79)	<0.01
Peritoneal catheter	2 (0.86%)	10 (2.46%)		0.16
Peripherical catheter	93 (39.91%)	170 (41.77%)		0.65
Admitting diagnosis				
Burn	15 (6.44%)	7 (1.72%)	1.93 (1.43–2.63)	< 0.01
Immunosuppression	5 (2.15%)	20 (4.91%)		0.08
Renal failure	27 (11.59%)	39 (9.58%)		0.42
Cardiovascular diseases	31 (13.3%)	81 (19.9%)	0.92 (0.56–1.51)	0.03
Malignancy	4 (1.72%)	13 (3.19%)		0.26
Diabetes mellitus	11 (4.72%)	21 (5.16%)		0.81
Neurological diseases	58 (24.89%)	79 (19.41%)		0.1
Chronic pulmonary disease	3 (1.29%)	13 (3.19%)		0.14
Comorbidities				
Renal failure	31 (13.3%)	36 (8.85%)		0.08
Cardiovascular diseases	87 (37.34%)	172 (42.26%)		0.22
Malignancy	7 (3%)	18 (4.42%)		0.34
Diabetes mellitus	61 (26.18%)	88 (21.62%)		0.18
Neurological diseases	26 (11.16%)	43 (10.57%)		0.82
Chronic pulmonary disease	8 (3.43%)	15 (3.69%)		0.87
Para/hemi/quadriplegia	2 (0.86%)	13 (3.19%)		0.87
Connective tissue disease	5 (2.15%)	2 (0.49%)	1.98 (1.23–3.20)	0.05
Immunosuppression	11 (4.72%)	22 (5.41%)		0.7
Antimicrobials used prior to isolation				
Ampicillin/sulbactam	72 (30.9%)	105 (25.8%)		0.17
Cephalosporins	159 (68.24%)	170 (41.77%)		0.93
Aztreonam	1 (0.43%)	4 (0.98%)		0.44
Fluoroquinolone	28 (12.02%)	57 (14%)		0.48
Piperacillin/tazobactam	74 (31.76%)	115 (28.26%)		0.35
Aminoglycosides	4 (1.72%)	13 (3.19%)		0.27
Trimethoprim/sulfamethoxazole	11 (4.74%)	14 (3.44%)		0.41
Carbapenems	159 (48.3%)	170 (51.7%)	2.03 (1.62–2.56)	< 0.01
Metronidazole	14 (6.03%)	25 (6.14%)		0.96
Linezolid	11 (4.74%)	15 (3.69%)		0.42
Vancomycin	145 (62.23%)	130 (32.02%)	2.18 (1.76–2.7)	< 0.01
Macrolide	17 (7.3%)	12 (2.95%)	1.66 (1.19–2.29)	0.01

CPE: carbapenem-producing *Enterobacterales*. APACHE II: Acute Physiology and Chronic Health Evaluation II score. ICU: intensive care unit; *Median, IQR: interquartile range.

Cases were defined as symptomatic or asymptomatic patients aged ≥18 years with CPE colonization or infection during the study. CPE infection was defined according to an established protocol from the Centers for Disease Control and Prevention (CDC) [6]. All asymptomatic carriers were considered colonised. Patients negative for CPE based on rectal and clinical samples and who remained negative throughout the study were considered controls. Patients were followed until discharge or death. Baseline data were collected for each individual using a questionnaire, including age, gender, diagnosis on admission, comorbidities, antimicrobial use prior to CPE isolation, and invasive devices. The ethical committee of The Catholic University of Santiago of Guayaquil approved the protocol [UCSG-CBICS-IED-2015-016]. Written informed consent was obtained from every patient or their relatives.

Microbiology

A total of 1,146 samples were collected from enrolled patients throughout the study. Samples were screened for carbapenem-resistant *Enterobacterales* (CRE) according to the CDC and CHROMagar mSuper CARBA agar (CHROMagar™) methods. CRE was defined according to the Clinical and Laboratory Standards Institute (CLSI) breakpoints [7]. *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Klebsiella pneumoniae* ATCC BAA-1705 were used as quality control strains. A modified carbapenemase inactivation method was used to identify carbapenamase production. All CRE were subcultured on trypticase soy agar (Oxoid, Basingstoke, UK) for 24 hours at 35°C in air. DNA was extracted using the Wizard Genomic DNA Purification Kit (Promega, São Paulo, Brazil) following the manufacturer's recommendations. DNA quality was assessed by analysing the ratio of absorbance at 260 nm/280 nm. Carbapenemase production was confirmed by the detection of the bla_{KPC}, bla_{OXA-48}, bla_{VIM}, bla_{IMP}, and bla_{NDM} genes by a multiplex polymerase chain reaction, as described previously, using a thermal cycler (BioRad, Hercules, CA, USA). The reaction conditions were as follows: initial denaturation at 94°C for 10 min, followed by 35 cycles of denaturation at 94°C for 30 s, annealing at 52°C for 30 s, extension at 72°C for 1 min, and a final extension at 72°C for 10 min. The PCR products were resolved by 1% agarose gel electrophoresis at 120 V for 30 min. The gel was stained with Syber Safe (Invitrogen, Carlsbad, CA, USA). Primers used for the reactions have been described previously [8].

Statistical analysis

Data analyses were performed using R version 3.6.2 (Vienna, Austria: R Foundation for Statistical Computing, 2019; available at: www.R-project.org). Chi-squared tests or Fisher's exact tests and Student's *t*-tests or Mann–Whitney *U* tests were used to compare categorical or continuous variables, respectively. Multicollinearity, Pearson's correlation coefficients, and variance inflation factors for the logistic regression model were analysed. The Wald test was used with a significance level of $\alpha = 0.05$ and 95% confidence intervals (CIs) for the OR were obtained.

Results

Out of 640 patients enrolled in the study, 233 (36.4%) had at least one sample positive for CPE. *Klebsiella pneumoniae* was identified in 90.65% (N = 223), *Proteus mirabilis* in 2.85% (N = 7), *Enterobacter cloacae* in 2.44% (N = 6), *Escherichia coli* in 1.63% (N = 4), *Klebsiella aerogenes* in 1.2% (N = 3), and *Klebsiella oxytoca* in 1.22% (N = 3) of cases. Some patients had more than type of *Enterobacterales*. The Carbapenemase types were *K. pneumoniae* carbapenemase (KPC) (97.15%, N = 239) and New Delhi Metallo-beta-lactamase (NDM) (2.84%, N = 7). Clinical and epidemiologic characteristics of patients and antimicrobial use are provided in Table 1.

Risk factors for infection or colonisation with CPE before current admission in patients who tested positive after enrolment included previous hospitalisation (RR 1.44; 95% CI 0.86–1.58; $p < 0.01$), use of invasive procedures (RR 1.17; 95% CI 0.86–1.58; $p = 0.33$), haemodialysis (RR 1.05; 95% IC 0.60–1.83; $p = 0.87$), long-term care (RR 0.6; 95% CI 0.13–3.75; $p = 0.63$), immunosuppression (RR 0.85; 95% CI 0.49–1.47; $p =$

Table 2. Multivariate analysis of risk factors for carbapenemase-producing *Enterobacterale*s colonisation/infection.

Characteristic	qOR (CI 95%)	*p*-value
Long ICU stay	1.05 (1.03–1.08)	< 0.01
Tracheostomy	3.52 (1.90–6.75)	< 0.01
Hospitalisation 3 months prior to admission	2.07 (1.17–3.71)	< 0.01
Vancomycin administration	3.31 (2.02–5.18)	< 0.01
Macrolide administration	3.31 (1.43–7.86)	< 0.01

0.54), and antimicrobial use (RR 1.18; 95% CI 0.91–1.54; $p = 0.23$) (Table 2).

Discussion

Prospective studies aimed at identifying risk factors for CPE colonisation/infection in patients in developing countries are scarce. The incidence and prevalence of CPE varies among geographical regions. Previous studies have reported values ranging for 0.6% in Asia to 35.2% in Uganda. In this study, we observed an incidence of 36.4%, which is higher than those in other countries in South America, like Brazil [9,4]. The length of ICU stay and invasive device use contribute to CPE infection [3,10], consistent with the results of this study. Tracheostomy was the only invasive device associated with CPE colonisation/infection in our multivariate analysis, which is inconsistent with the results of some previous studies [11,12]. Therefore, additional studies of ventilator-associated pneumonia as an important end point are needed. Carbapenems, fluoroquinolones, antipseudomonal penicillin, and broad-spectrum cephalosporins increase the risk of KPC colonisation [3]. We were unable to confirm that the administration of these drugs is associated with CPE based on a multivariate analysis. Fluoroquinolones and broad-spectrum cephalosporins are not frequently used in the hospitals studied; thus, a limited number of patients received these antimicrobials, making statistical analyses difficult. We obtained two unexpected results: i) vancomycin administration increased CPE-colonisation two-fold and ii) the presence of macrolides was a risk factor. Vancomycin induces drastic and consistent changes in the human intestinal microbiota, with a decrease in gram-positive bacteria (mainly Firmicutes) and a compensatory increase in gram-negative bacteria. Importantly, several genera belonging to the phylum Proteobacteria (i.e., *Escherichia/Shigella* and *Klebsiella*) increase after vancomycin administration [13,14]. This effect could explain the detection of CPE in ICU patients exposed to a short course of vancomycin treatment. The identification of macrolides as risk factors can be explained by the fact that azithromycin treatment can increase the selection of macrolide resistance genes in the gut microbiome [15], which acts as an important reservoir of various resistance genes. Different mechanisms underlying macrolide resistance have been identified in *Enterobacterales*, some of which can be transferred amongst microorganisms via mobile genetic elements. Transferable mechanisms of macrolide resistance (TMMR) are present in genetic structures that also include β-lactam resistance; for example, the IncH1 plasmid from *C. freundii,* which might also be involved in macrolide extrusion, can harbour the $bla_{\text{NDM-1}}$ gene [16]. Detailed studies of these resistance mechanisms are needed to better understand the relationship between macrolides and CPE.

The discovery of macrolide use as a risk factor has critical implications, since clarithromycin is a first-line treatment in severe community-acquired pneumonia and vancomycin is included as part of the empiric treatment in septic shock and high-risk febrile neutropenia [17]. Another macrolide, azithromycin, was initially considered for the treatment of COVID-19-related pneumonia and the collateral effects of this drug should be carefully addressed during the pandemic [18]. Our results confirm a report by the World Health Organization in 2017, suggesting that the use of these drugs should be prioritised for monitoring as part of an appropriate antimicrobial stewardship program [17]; these programs are scarce in developing countries, such as Ecuador. Thus, modifying the recommended empirical antimicrobial treatments based on the infectious diseases processes of each patient and improving our understanding of the clinical and molecular epidemiological properties as well as resistance mechanisms are key goals. Patients colonised or infected with CPE in our study had a significantly higher mortality rate than that of controls. This is an important finding, since most studies have focused on infection and its relationship with higher mortality rates. In one study, colonisation did not increase ICU mortality, whereas KPC infection did [11]. Our findings require further research for validation and highlight the importance of early identification and isolation [19]. The difficulty in obtaining reliable information on antimicrobial exposure and the use of invasive procedures outside of the study centres was a limitation of our study. We did not collect information about other microorganisms and related infections, which could result in selection bias. Finally, the short study period and the composite of variables related to colonisation and infection could have affected the identification of factors that determine the shift from colonisation to infection.

Conclusions

Although risk factors associated with CPE have been studied extensively in developed countries, it is imperative to evaluate these factors in developing countries with different public health contexts. Our study contributes to the few reports indicating that tracheostomy use as well as vancomycin and macrolide administration are risk factors for CPE, at least in

developing countries. This information highlights the importance of including macrolide and vancomycin as WATCH antibiotics in antimicrobial stewardship programs and the necessity of reviewing empirical treatment recommendations. In this sense, there is a need for more research to improve antimicrobial stewardship programs and ultimately to prevent the spread of CPE.

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Corresponding authors

Carmen, Soria-Segarra MD. MsC
Instituto de Innovación y Salud Integral, Universidad Católica Santiago de Guayaquil
Av. Pdte. Carlos Julio Arosemena Tola Km 1 ½ . EC090615. Guayaquil, Ecuador.
Department of Microbiology, School of Medicine and PhD Program in Clinical Medicine and Public Health, University of Granada
Campus Universitario de Fuentenueva, Avenida de Fuentenueva s/n, 18071 Granada, Andalucia, España
Tel: +5939 97417005
E-mail: carmen.soria01@cu.ucsg.edu.ec.

Claudia Soria-Segarra MD. MsC
Faculty of Medical Sciences, Universidad de Guayaquil.
Avenida 10 NO, EC 90613, Guayaquil, Ecuador
Tel: +593 42284505
Laboratorio Cínico "SOSEGAR
Pedro Moncayo 2929 y Francisco de Marcos
Tel: +593 2411757
E-mail: claudia.sorias@ug.edu.ec

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