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Molecular epidemiology of carbapenem-resistant gram-negative bacilli in Ecuador

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Abstract

Introduction Carbapenem-resistant gram-negative bacilli are a worldwide concern because of high morbidity and mortality rates. Additionally, the increasing prevalence of these bacteria is dangerous. To investigate the extent of antimicrobial resistance and prioritize the utility of novel drugs, we evaluated the molecular characteristics and antimicrobial susceptibility profiles of carbapenem-resistant Enterobacterales, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in Ecuador in 2022.

Methods Ninety-five clinical isolates of carbapenem non-susceptible gram-negative bacilli were collected from six hospitals in Ecuador. Carbapenem resistance was confirmed with meropenem disk diffusion assays following Clinical Laboratory Standard Institute guidelines. Carbapenemase production was tested using a modified carbapenemase inactivation method. Antimicrobial susceptibility was tested with a disk diffusion assay, the Vitek 2 System, and gradient diffusion strips. Broth microdilution assays were used to assess colistin susceptibility. All the isolates were screened for the bla_{KPC}, bla_{NDM}, bla_{OXA-48}, bla_{VIM} and bla_{IMP} genes. In addition, *A. baumannii* isolates were screened for the bla_{OXA-23}, bla_{OXA-58} and $bla_{OXA-24/40}$ genes.

Results Carbapenemase production was observed in 96.84% of the isolates. The bla_{KPC}, bla_{NDM} and bla_{OXA-48} genes were detected in Enterobacterales, with bla_{KPC} being predominant. The bla_{VIM} gene was detected in *P. aeruginosa,* and $bla_{OXA-24/40}$ predominated in *A. baumannii*. Most of the isolates showed co-resistance to aminoglycosides, fluoroquinolones, and trimethoprim/sulfamethoxazole. Both ceftazidime/avibactam and meropenem/vaborbactam were active against carbapenem-resistant gram-negative bacilli that produce serin-carbapenemases.

Conclusion The epidemiology of carbapenem resistance in Ecuador is dominated by carbapenemase-producing *K. pneumoniae* harbouring bla_{KPC}. Extensively drug resistant (XDR) *P. aeruginosa* and *A. baumannii* were identified, and their identification revealed the urgent need to implement strategies to reduce the dissemination of these strains.

Keywords Carbapenem resistance, Carbapenemase genes, Ecuador, Gram-negative bacilli

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Introduction

The increasing number of multidrug-resistant organisms (MDROs) constitutes a major threat to health worldwide. MDROs are linked to increasing costs and mortality rates [1] and are an important challenge to clinicians due to the complicated choice of treatment option [2].

The World Health Organization included carbapenem-resistant *Acinetobacter baumannii, Pseudomonas aeruginosa*, and Enterobacterales as "priority antibiotic-resistant pathogens", for which the research and development of new antimicrobial agents is critical [3]. The dissemination of these pathogens is mainly due to high-risk clones and is generally associated with health care-associated infections with high mortality rates [4, 5].

The mechanisms of carbapenem resistance are diverse but are dominated by the expression of carbapenemases. The prevalence of carbapenemases varies according to region [2]. In Ecuador, despite the high prevalence of carbapenem-resistant Enterobacterales (CRE) (37%) and other gram-negative rod-shaped bacteria [6, 7], there is little information about the mechanisms involved and the antimicrobial susceptibility profiles, including that to recently approved antibiotics such as ceftazidime/avibactam, meropenem/vaborbactam, or ceftolozane/tazobactam, which constitute the first line of treatment against carbapenem-resistant gram-negative rod-shaped bacteria [8, 9].

Thus, essential information is required to optimize the administration of antibiotics as a lack of knowledge could lead to the misuse of antibiotics and the rapid development of antimicrobial resistance.

Therefore, to clarify the extent of antimicrobial resistance and prioritize the utility of newly available drugs, our goal was to determine the molecular characteristics and antimicrobial susceptibility profiles of clinical CRE, *P. aeruginosa* and *A. baumannii* complex isolates from Ecuador.

Methodology

A multicentre study was carried out in six hospitals in Ecuador between January 2022 and May 2022. Clinical isolates of CRE, *P. aeruginosa* and the *A. baumannii* complex were collected according to protocols established by each institution. Carbapenem resistance was defined when the isolate was non-susceptible to any of the carbapenems tested according to Clinical Standards Institute (CLSI) breakpoints [10]. Only one clinical isolate from each patient was studied, and there was a preference for samples from sterile sites or those with the greatest resistance phenotype.

CRE isolates were cultured on CHROMagar Super Carba (CHROMagar, France). *P. aeruginosa* and *A. baumannii* complex isolates were cultured on MacConkey agar (16–18 h; 35 °C) (Becton–Dickinson, England) to check their viability and purity. Isolates were identified with the Vitek 2 System (BioMérieux, France) and conventional biochemical tests.

Carbapenem resistance was confirmed with meropenem disk diffusion assays using CLSI methodology. Carbapenemase production was studied with the modified carbapenem inactivation method (mCIM) for Enterobacterales and *P. aeruginosa* according to CLSI guidelines [10]. *A. baumannii* complex isolates were studied with the optimized carbapenem inactivation method described by Zhang S. et al. [11].

Antimicrobial susceptibility testing

Susceptibility tests for ciprofloxacin, amikacin, gentamicin, trimethoprim/sulfamethoxazole, tigecycline, ceftazidime, cefepime, meropenem imipenem, ampicillin/sulbactam and piperacillin/tazobactam were performed using disk diffusion assays and the Vitek 2 System (AST-N402 or AST-N401 Card) (BioMérieux, France). Minimal inhibitory concentrations (MICs) for ceftazidime/avibactam and meropenem/vaborbactam were determined using gradient diffusion strips (Liofilchem, Italy). MIC values and disk diffusion results were interpreted using CLSI breakpoints [10]. For tigecycline, the U.S. Food and Drug Administration (FDA) breakpoints were used (susceptible $\geq$ 19 mm or $\leq$ 2 μg/ml) for Enterobacterales [12]. For meropenem/vaborvactam, European Committee on Antimicrobial Susceptibility (EUCAST) testing breakpoints were used for *P. aeruginosa* (susceptible $\leq$ 8 μg/ml) [13].

Susceptibility to ceftazidime/avibactam and meropenem/vaborbactam was not tested in NDM-positive isolates.

The MIC of colistin was obtained using a broth microdilution (CBM) method, as described in CLSI document M07-A8 [14]. Analytical grade colistin sulfate (Sigma–Aldrich Code C2700000, batch 3.0) and Mueller Hinton broth with cation adjustment (Thermo Fischer Scientific, United Kingdom) were used. The concentration range was 0.5–4 μg/mL, and CLSI breakpoints were used to define colistin resistance (MIC values $\geq$ 4 μg/mL) [10]. The MIC_{50} and MIC_{90} were determined for each antimicrobial.

Escherichia coli ATCC 25922, *P. aeruginosa* ATCC 27853, *K. pneumoniae* BAA ATCC 1705 and *E. coli* AR Bank #0349 were used for quality control.

Detection of carbapenemase and mcr-1 genes

The carbapenemase-encoding genes $bla_{KPC,}$ $bla_{NDM,}$ $bla_{OXA-48,}$ $bla_{VIM,}$ and bla_{IMP} were studied using multiplex polymerase chain reaction (PCR) in all isolates [15]. Additionally, the $bla_{OXA-23-like}$, bla_{OXA-58} and

$bla_{\text{OXA-24/40-like}}$ genes were studied in *A. baumannii* complex isolates with a previously described multiplex PCR method [16].

The molecular detection of the colistin-resistance gene *mcr-1* was performed via PCR [17].

Clonality study

Clonal relatedness was studied in bla_{KPC}-positive *K. pneumoniae,* bla_{VIM}-positive *P. aeruginosa* and $bla_{\text{OXA-24/40}}$-positive *A. baumannii* complex using ERIC-PCR following a previously described method [18].

Dendrograms were constructed using GelJ software based on Dice's similarity coefficient and the unweighted pair group method (UPGM) (tolerance 2%). The cut-off level for ERIC-PCR electrophoretic pattern delineation was 80% similarity.

Results

One hundred and twenty-nine isolates were obtained for analysis. Thirty-four were rejected for inconsistencies in shipments or duplications (26.35%). Thus, a total of ninety-five isolates were included in the study (73.64%).

Enterobacterales predominated among the sample and accounted for 60 isolates studied (63.15%), with *K. pneumoniae* being the most frequently isolated species ($n=45$; 47.36%). *K. aerogenes, E. cloacae* and *E. coli* were also present in 16.66% ($n=10$), 5% ($n=3$) and 3.33% ($n=2$) of samples, respectively. The *A. baumannii* complex was present in 25.26% ($n=24$) of the samples, and *P. aeruginosa* was present in 11.57% ($n=11$) of the samples.

Carbapenemase production was confirmed in 92 isolates (96.84%). Three *P. aeruginosa* isolates tested negative for mCIM.

Bla_{KPC} was the most frequent carbapenemase-encoding gene found in Enterobacterales ($n=52$; 86.66%). Bla_{NDM} ($n=7$; 11.66%) and $bla_{\text{OXA-48}}$ ($n=1$; 1.66%) were also detected. Neither bla_{IMP} nor bla_{VIM} was detected in Enterobacterales. Both ceftazidime/avibactam (MIC_{50} and $MIC_{90} <= 0.12/4$ µg/ml) and meropenem/vaborbactam (MIC_{50} 0.064/8 µg/ml; MIC_{90} 0.84/8 µg/ml) were active against all tested isolates harbouring bla_{KPC}. The susceptibility rates to CPE were 95% and 76.67% for tigecycline and colistin, respectively.

Detailed information about the susceptibility patterns of the CPE strains, *K. pneumoniae* strains *and K. aerogenes* bla_{KPC} strains is provided in Table 1.

Tigecycline and colistin were the only drugs that *K. aerogenes* bla_{NDM} isolates were susceptible to ($n=1$). *E. cloacae* bla_{KPC} isolates ($n=2$) were resistant to most of the antimicrobials tested, except for amikacin tigecycline, colistin, ceftazidime/avibactam and meropenem/vaborbactam, as observed for *E. coli* bla_{KPC} ($n=1$).

E. cloacae bla_{NDM} ($n=1$) was only susceptible to colistin and amikacin.

The susceptibility profile of *E. coli* $bla_{\text{oxa-48}}$ was the most conserved. This isolate was resistant to only ciprofloxacin, trimethoprim/sulfamethoxazole, and ceftazidime. Tigecycline, colistin, ceftazidime/avibactam, trimethoprim/sulfamethoxazole and colistin were active against *E. coli* $bla_{\text{KPC.}}$ These strains were resistant to ciprofloxacin, aminoglycosides and all beta-lactam antimicrobial agents tested.

In *P. aeruginosa,* only bla_{VIM} was detected ($n=8$; 72.72%*).* Three isolates lacked any of the examined carbapenemase genes and tested negative for mCIM. All antimicrobials tested showed high resistance in bla_{VIM}-positive *P. aeruginosa*. Ceftolozane/tazobactam was active against carbapenemase-negative *P. aeruginosa*.

In *A. baumannii* complex isolates, 66.66% ($n=16$) of the patients were $bla_{\text{OXA-24/40}}$ positive, and 33.33% ($n=8$) of the patients were bla_{OXA23} positive. $bla_{\text{KPC,}}$ $bla_{\text{NDM,}}$ $bla_{\text{OXA-48,}}$ $bla_{\text{VIM,}}$ and bla_{IMP} were not detected in any of the isolates. *A. baumannii* complex isolates were only susceptible to colistin (95%), regardless of which gene was present in the isolates.

Tables 2 and 3 provide comprehensive details regarding carbapenem-resistant non-fermentative gram-negative pathogens. The MIC values of the antimicrobials evaluated are detailed in Supplementary Table 1.

None of the colistin-resistant isolates carried the *mcr-1* gene.

$Bla_{\text{VIM-}}$positive *P. aeruginosa* and $bla_{\text{KPC-}}$positive *K. pneumoniae* ($n=35$) isolates exhibited significant genetic heterogenicity (Supplementary Figs. 1 and 2).

In the $bla_{\text{OXA-24/40-}}$positive *A. baumannii* complex, six electrophoretic patterns were found, and 50% of the isolates belonged to only one pattern, indicating the clonal dissemination of this microorganism (Supplementary Fig. 3).

Four electrophoretic patterns were found in $bla_{\text{KPC-}}$positive *K. aerogenes* (8 isolates studied) (Supplementary Fig. 4).

Discussion

Our research describes the molecular characteristics of carbapenem-resistant gram-negative rod-shaped bacteria in Ecuador. Carbapenemase production was prevalent in the isolates studied (96.64%), and carbapenemase production has been described by several authors as the main mechanism involved in carbapenem resistance [6, 19–21].

Carbapenemase-encoding genes such as bla_{KPC}, bla_{NDM} and $bla_{\text{OXA-48}}$ were detected in Enterobacterales, and bla_{KPC} was the predominant gene. Our results are similar to those described in other Western countries, such

Table 1 Antimicrobial susceptibility against carbapenemase-producing Enterobacterales

Antimicrobials	CPE ($n=60$)		*K. pneumoniae* bla_{KPC} ($n=40$)				*K. pneumoniae* bla_{NDM} ($n=5$)				*Klebsiella aerogenes* bla_{KPC} ($n=9$)			
	Susceptible		Susceptible		MIC_{50} μg/ml	MIC_{90} μg/ml	Susceptible		MIC_{50} μg/ml	MIC_{90} μg/ml	Susceptible		MIC_{50} μg/ml	MIC_{90} μg/ml
	No	%	No	%			No	%			No	%		
AK	27	45.00	21	47.73	4	≥64	0	0.00	≥64	≥64	2	22.22	≥64	≥64
GN	11	18.33	8	18.18	≥64	≥64	0	0.00	≥64	≥64	2	22.22	≥64	≥64
CIP	8	13.33	5	11.36	≥4	≥4	1	20.00	≥4	≥4	2	22.22	≥4	≥4
SXT	4	6.67	4	9.09	4/76	4/76	0	0.00	4/76	4/76	0	0.00	4/76	4/76
TIG	57	95.00	38	86.36	≤0,5	≤0,5	5	100.00	2	2	9	100.00	≤0.5	≤0.5
COL	46	76.67	34	85.00	1	≥8	5	100.00	1	1	1	11.11	≥8	≥8
CAZ/AVI	53[a]	100.00	40	100.00	≤0.12/4	≤0.12/4	NT	NT	NT	NT	9	100.00	DD	DD
MER/VAB	25[b]	100.00	23[c]	100.00	0.064/8	0.94/8	NT	NT	NT	NT	1[d]	100.00	-	-
CAZ	0	0.00	0	0.00	≥64	≥64	0	0	≥64	≥64	0	0.00	≥64	≥64
FEP	1	0.00	0	0.00	≥32	≥32	0	0	≥32	≥32	0	0.00	≥32	≥32
IMP	1	3.33	0	0.00	≥16	≥16	0	0	≥16	≥16	0	0.00	≥16	≥16
MER	1	3.33	0	0.00	≥16	≥16	0	0	≥16	≥16	0	0.00	≥16	≥16
PTZ	0	0.00	0	0	≥128/4	≥128/4	0	0	≥128/4	≥128/4	0	0.00	≥128/4	≥128/4

NT not tested, *DD* disk diffusion, *AK* Amikacin, *GN* Gentamicin, *CIP* Ciprofloxacin, *SXT* Trimethoprim/sulfamethoxazole, *TIG* Tigecycline, *COL* Colistin, *CAZ/AVI* Ceftazidime/Avibactam, *MER/VAB* Meropenem/vaborbactam, *CAZ* Ceftazidime, *FEP* Cefepime, *IMP* Imipenem, *MER* Meropenem, *PTZ* Piperacillin/tazobactam

[a] 53 isolates tested

[b] 25 isolates tested

[c] 23 isolates tested

[d] 1 isolate tested

Table 2 Susceptibility antimicrobial against *P. aeruginosa*

Antimicrobials	*P aeruginosa* bla_{VIM} $n=8$				*P.aeruginosa* mCIM negative $n=3$				*P. aeruginosa* $n=11$	
	Susceptible		MIC_{50} µg/ml	MIC_{90} µg/ml	Susceptible		MIC_{50} µg/ml	MIC_{90} µg/ml	Susceptible	
	No	%			No	%			No	%
AK	4	50	4	32	2	66.67	16	16	6	54.54
GN	2	25	≥32	≥32	1	33.33	4	8	3	27.27
CIP	1	12.5	≥4	≥4	1	33.33	0.5	≥4	1	9.09
COL	4	50	2	≥8	3	100.00	1	1	7	63.63
CAZ/AVI	NT	NT	NT	NT	3[a]	100.00	DD	DD	3[a]	100
MER/VAR	NT	NT	NT	NT	3[a]	100.00	0.5	1	3[a]	100
CAZ	0	0	≥64	≥64	2	66.67	4	≥64	2	18.18
FEP	0	0	≥32	≥32	1	33.33	8	≥32	1	9.09
IMP	0	0	≥16	≥16	0	0.00	≥16	≥16	0	0
MER	0	0	≥16	≥16	0	0.00	≥16	≥16	0	0
CEF/TAZ	0	0	>256	>256	3	100.00	1.5	4	3	27.27
PTZ	0	0	≥128/4	≥128/4	0	0.00	≥128/4	≥128/4	0	0

NT not tested, *DD* Disk diffusion, *AK* Amikacin, *GN* Gentamicin, *CIP* Ciprofloxacin, *COL* Colistin, *CAZ/AVI* Ceftazidime/avibactam, *MER/VAB* Meropenem/vaborbactam, *CAZ* Ceftazidime, *FEP* Cefepime, *IMP* Imipenem, *MER* Meropenem, CEF/TAZ, *PTZ* Piperacillin/tazobactam

[a] EUCAST Breakpoints

Table 3 Susceptibility antimicrobial against *A. baumannii* complex

Antimicrobials	*A.baumannii* complex bla_{OXA23} $n=8$				*A.baumannii* complex $bla_{OXA24/40}$ $n=16$				*A. baumannii* complex $n=24$	
	Susceptible				Susceptible				Susceptible	
	No	%	MIC_{50} µg/ml	MIC_{90} µg/ml	No	%	MIC_{50} µg/ml	MIC_{90} µg/ml	No	%
AK	0	0.00	≥64	≥64	0	0.00	≥64	≥64	0	0
GN	0	0.00	≥64	≥64	0	0.00	≥64	≥64	0	0
CIP	0	0.00	≥4	≥4	0	0.00	≥4	≥4	0	0
SXT	0	0.00	4/76	4/76	0	0.00	4/76	4/76	0	0
COL	6[a]	100.00	1	1	13[b]	92.86	1	1	19±	95
CAZ	2	25.00	≥64	≥64	2	12.50	≥64	≥64	4	18.18
FEP	0	0.00	≥32	≥32	0	0.00	16	≥32	0	0
IMP	0	0.00	≥16	≥16	0	0.00	≥16	≥16	0	0
MERO	0	0.00	≥16	≥16	0	0.00	≥16	≥16	0	0
PTZ	0	0.00	≥128/4	≥128/4	0	0.00	≥128/4	≥128/4	0	0
SAM	0	0.00	≥32/16	≥32/16	2	12.50	≥32/16	≥32/16	2	8.33

AK Amikacin, *GN* Gentamicin, *CIP* Ciprofloxacin, *SXT* Trimethoprim/sulfamethoxazole, *COL* Colistin, *CAZ* Ceftazidime, *FEP* Cefepime, *IMP* Imipenem, *MER* Meropenem, *PTZ* Piperacillin/tazobactam, *SAM* Ampicillin/sulbactam

[a] 6 isolates tested

[b] 14 isolates tested ± 20 isolates tested

as the United States, Argentina, Colombia, and Brazil, which described bla_{KPC} as the prevalent gene linked to *K. pneumoniae* [7, 21, 22]. Our results also agree with previous data published in 2022 by the National Reference Laboratory, which described *K. pneumoniae* harbouring bla_{KPC} as the main cause of carbapenem resistance in Enterobacterales in Ecuador [23].

In our study, we did not find bla_{NDM} (11.47%) or bla_{OXA-48} (1.64%) genes very frequently in Enterobacterales; these percentages are comparable to those reported in a national surveillance report [23]. However, given the small number of isolates, we advise using caution when considering this information. Bla_{NDM} was detected in *K. pneumoniae, K. aerogenes* and *E. cloacae.* Interestingly,

we did not find any *P. rettgeri* isolates harbouring this gene because this species has been shown to play a central role in the dissemination of bla_{NDM} in Latin America, but infections by *P. rettgeri* are not very common [24]. Similar to other studies, we found bla_{OXA-48} only in *E. coli*, which is the main species associated with this gene [25, 26].

The pattern observed in Ecuador is different from that described in other countries, such as India, where *NDM* is prevalent, or Turkey, where *OXA-48* dominates among CRE [26, 27].

During the COVID-19 pandemic, an increase in carbapenemase-producing Enterobacterales was observed; moreover, the emergence of isolates harbouring more than one carbapenemase gene was reported [28]. In 2021, Ecuador reported $bla_{KPC} + bla_{NDM}$ and $bla_{KPC} + bla_{OXA-48}$ associations [28, 29]. Nevertheless, we did not find any combinations of carbapenemase genes in any of the isolates studied.

Co-resistance to aminoglycosides and fluoroquinolones was observed in *K. pneumoniae* bla_{KPC}-positive isolates. This association has also been described by several authors and is linked to horizontal dissemination [30, 31]. Genetic diversity was revealed in our isolates, suggesting horizontal dissemination. Interestingly, our results differ from those of previous research in which clonal circulation of *K. pneumoniae* bla_{KPC}-positive isolates was documented [18]. The differences found could be attributed to the fact that our study was not limited to one city or hospital area, such as intensive care units, where most other research is focused.

According to our results, tigecycline could be considered a therapeutic alternative for Enterobacterales isolates, independent of carbapenemase genes or the species involved, but its use will require a previous antimicrobial susceptibility report, as other authors have also suggested [32].

Enterobacterales harbouring bla_{KPC} showed reduced susceptibility to colistin, but surprisingly, all isolates harbouring bla_{NDM} remained susceptible to colistin, contrary to the findings of other authors, who described an increase in colistin resistance in CRE isolates harbouring bla_{NDM} [33]. Based on our findings, colistin may be considered a significant therapeutic option for the treatment of CRE, but a prior susceptibility report may be needed. The *mcr*-1 gene was not detected in any of our isolates, in contrast with the results of other Latin American studies [34–36], suggesting that resistance could be mediated by chromosomal mutations or other *mcr* variants that were not studied and have been described in the region, including *mcr-3* or *mcr-5* [37–39]. Further studies are needed to understand the resistance mechanisms involved in colistin resistance.

New antimicrobial agents, such as ceftazidime/avibactam and meropenem/vaborbactam, demonstrated complete susceptibility in all the bla_{KPC}-positive isolates. Despite the susceptibility patterns reported by several authors, the emergence of KPC isolates with ceftazidime/avibactam resistance has been reported, which has been frequently associated with mutations leading to substitutions in the Ω-loop of the KPC-3 variant [40, 41]. Fortunately, our research did not show any resistance to this new antimicrobial in KPC isolates, perhaps due to the recent introduction of this antimicrobial (June 2022) in Ecuador, although some cases of CAZ/AVI resistance have been reported prior to drug introduction in other countries through a salt bridge between glutamic 197 and arginine 164 in the wild-type KPC enzyme; however, this result was not found in this study [42].

Carbapenemase production by the bla_{VIM} gene was detected in 63.63% of *P. aeruginosa* isolates, and this gene encodes a metallo-β-lactamase. Our findings are consistent with several other authors who determined that this gene was prevalent [43]. *P. aeruginosa* exhibited high rates of co-resistance to aminoglycosides, ciprofloxacin, colistin and ceftolozane/tazobactam, rendering these antimicrobials ineffective for empirical treatment. Our findings differ from those of other authors who recommended ceftolozane/tazobactam as an effective treatment for multidrug-resistant *P. aeruginosa* [44]. In contrast to our findings, *Ajila* et al. reported good susceptibility to ceftolozane/tazobactam in *P. aeruginosa* isolates recovered in 2019 in Ecuador [45]. We attributed these conflicting findings to the molecular mechanisms involved in the studied isolates, but these hypotheses were not described by the authors.

In America, carbapenem resistance in *A. baumannii* complex isolates is principally mediated by oxacillinases, particularly bla_{OXA-23} and bla_{OXA-58} [46, 47]. In 2016, Nuñez-Quezada et al. reported an outbreak of a carbapenem-resistant *A. baumannii* complex with bla_{OXA-72}, a member of the $bla_{OXA-24/40}$ subgroup, in Guayaquil, Ecuador [48]. Our research revealed that $bla_{OXA-24/40}$ (66.66%) was predominant in *A. baumannii* complex isolates, similar to the results of a previous report in our country; furthermore, the same PCR pattern was observed in 50% of the isolates, which indicates the clonal spread of this microorganism, as has been previously reported [48].

Nevertheless, our results differ from those published in a national surveillance report, which showed a predominance of *OXA-23* in *A. baumannii* complex isolates collected from 2019 to 2021. However, an increase in *OXA-24/40* was observed in 2021, with similar values to those of *OXA-23* (bla_{OXA-23} $n = 129$; $bla_{OXA-24/40}$ $n = 116$). In addition, we did not detect bla_{OXA-58} in our isolates, although it has been reported previously in Ecuador [23].

Our results also differ from the regional epidemiology [49], where the $bla_{\text{OXA-24/40}}$ gene has been described only sporadically and is mainly reported in countries such as Taiwan, China and South Korea in Asia [50, 51].

Co-resistance to other groups of drugs is highly common in *A. baumannii* complex isolates, and this co-resistance reduces the number of therapeutic options. The extensive drug resistance (XDR) phenotype was independent of the oxacillinase gene. The isolates only showed 100% susceptibility to colistin, and colistin could be considered a last resort option for these XDR isolates.

This study has several limitations. First, our results could not be generalized to primary care hospitals or other cities as the hospitals were not randomly selected; instead, they included hospitals from public and private services from the second and third levels of attention in Ecuador´s most populous cities. Second, clinical isolates of carbapenem-resistant gram-negative bacilli were selected by each microbiology laboratory according to their protocols and clinical requests for culture by physicians; therefore, some isolates were not included. Third, the sensitivity and specificity of the mCIM method are greater than 90%, although there could be false-negative results due to uncommon carbapenemase types that were not tested in our study [52]. Finally, bla_{OXA143}, which is an oxacillinase previously described in *A. baumannii* in Ecuador, has not been examined.

In conclusion, the epidemiology of carbapenem resistance in the three most important cities of Ecuador (Quito, Guayaquil, and Cuenca) was dominated by carbapenemase-producing *K. pneumoniae* harbouring bla_{KPC}. Additionally, XDR *P. aeruginosa* and *A. baumannii* complex isolates were also present, showing an urgent need to implement strategies to reduce their dissemination.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12879-024-09248-6.

Supplementary Material 1.

Supplementary Material 2.

Supplementary Material 3.

Supplementary Material 4.

Supplementary Material 5.

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Authors' contributions
SSCL, SSC, GFJ contributed to study conception, design, and analysis. SSCL wrote the draft version of the manuscript. MMM, AOI, NQT, CAK, MAM, STM, GHM, VHV, VJE, contributed to collection and data analysis. All authors commented on previous versions of the manuscript. All authors reviewed, revised, and approved the final manuscript.

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Availability of data and materials
The datasets used during the current study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate
The study was carried out according to the Declaration of Helsinki and was approved by the "Comite de Etica de Investigación en Seres humanos de la Pontificia Universidad Católica del Ecuador (PUCE) "[*EO-26–2021*]. Informed consent was waived due to the retrospective design of the study by the Ethical Committee of "Pontificia Universidad Católica del Ecuador (PUCE) (EO-26–20210.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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