BMC Microbiology

https://doi.org/10.1186/s12866-026-04940-w

Article in Press

Co-occurrence of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ genes in *Klebsiella pneumoniae* isolates from a tertiary-care hospital in Ecuador and evidence of emerging genotypic–phenotypic AMR inconsistencies

Received: 7 November 2025

Accepted: 6 March 2026

Published online: 18 March 2026

Cite this article as: Fernández-Salvatierra D., González-Domenech C.M., Morey-León G. *et al.* Co-occurrence of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ genes in *Klebsiella pneumoniae* isolates from a tertiary-care hospital in Ecuador and evidence of emerging genotypic–phenotypic AMR inconsistencies. *BMC Microbiol* (2026). https://doi.org/10.1186/s12866-026-04940-w

David Fernández-Salvatierra, Carmen María González-Domenech, Gabriel Morey-León & Juan Carlos Fernández-Cadena

Title

Co-occurrence of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ genes in *Klebsiella pneumoniae* isolates from a Tertiary-Care Hospital in Ecuador and evidence of emerging genotypic–phenotypic AMR inconsistencies

Author names and affiliations

David Fernández-Salvatierra[1], Carmen María González-Domenech[1,2,*], Gabriel Morey-Leon[3,*], Juan Carlos Fernández-Cadena[4,5]

[1]Department of Microbiology. School of Medicine. University of Malaga, 29010. Malaga, Spain.

[2]Clinical Research in HIV Infection, Endovascular Infection and Bacteriemia, Biomedical Research Institute of Malaga (IBIMA), 29010 Malaga, Spain.

[3]Ecotec University, Km. 13.5 Samborondón, Samborondón, EC092302, Ecuador

[4]Omics Sciences Laboratory, Faculty of Health Sciences, Espíritu Santo University, Samborondón, Ecuador

[5]Harvard Medical School and Brigham and Women´s Hospital. Boston, MA, United States

*Corresponding authors: gmorey@ecotec.edu.ec; Ecotec University, Km. 13.5 Samborondón, Samborondón, EC092302, Ecuador. Phone:

+45 150400 ext 634; cmgodo@uma.es; Department of Microbiology. School of Medicine. University of Malaga. Boulevard Louis Pasteur, 29010. Malaga, Spain. Phone: +34 952133211

Abstract

Background: Antimicrobial resistance (AMR) is a growing threat to public health, especially in low- and middle-income countries. This study aimed to identify multidrug-resistant (MDR) pathogens in clinical samples from a tertiary hospital in Ecuador, evaluate their phenotypic antibiotic susceptibility, and compare these findings with genotypic resistance patterns.

Methods: Clinical samples were collected from an Ecuadorian reference hospital from September 2022 to September 2023. Bacterial identification and susceptibility testing were performed using the VITEK-2 system. After genomic DNA extraction, key resistance genes (*bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, *bla*$_{CTX-M}$ and *bla*$_{IMP-1}$) were identified via multiplex PCR with TaqMan probes. Phenotypic and genotypic data were integrated using bioinformatics tools.

Results: The 216 bacterial isolates analyzed exhibited resistance phenotypes, primarily to carbapenems and third-generation cephalosporins. *Klebsiella pneumoniae* was the most prevalent resistant species (59.7%), followed by *Pseudomonas aeruginosa* (11.6%) and *Serratia marcescens* (8.3%). However, 20.8% of the isolates phenotypically resistant to carbapenems did not carry any of

the targeted carbapenemase genes. The *bla*$_{KPC}$ gene was most common (70.8%), followed by *bla*$_{NDM}$ (9.7%) and *bla*$_{VIM}$ (2.8%). Ten isolates harbored gene combinations, including, to our knowledge, the first reported co-occurrence of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ in *K. pneumoniae* in Ecuador.Neither *bla*$_{IMP-1}$ nor *bla*$_{CTX-M}$ was detected in any of the evaluated isolates.

Conclusions: There is strong concordance between carbapenemase genes and the MDR phenotype, some of which are newly reported in Ecuador. However, the absence of gene detection in some resistant isolates suggests the presence of alternative resistance mechanisms. Integrating phenotypic and molecular methods that target locally prevalent resistance genes improves the accuracy of AMR detection and supports better diagnosis and infection control in hospital settings

Keywords

Antimicrobial resistance, *Klebsiella pneumoniae*, carbapenemases, molecular diagnosis, Ecuador

Background

According to the American Centers for Disease Control and Prevention (CDC), antimicrobial resistance (AMR) occurs when microorganisms gain the ability to resist the drugs designed to eliminate them. This makes infections more difficult to treat, increasing the risk of disease spread, severe illness, and mortality [1]. Thus, AMR is a major emerging threat to global health, directly causing over 1 million deaths annually and contributing to nearly 5 million deaths per year. Additionally, it is estimated that AMR could cause nearly 2 million deaths and be associated with 8 million deaths globally in 2050 [2].

The World Health Organization (WHO) published a priority list of bacterial pathogens in 2017, which was updated in 2024 to identify the pathogenic bacteria posing the greatest public health threat associated with AMR [3,4]. This list includes *Escherichia coli,*

Staphylococcus aureus, Klebsiella pneumoniae, Streptococcus pneumoniae, Acinetobacter baumanii, Pseudomonas aeruginosa, Mycobacterium tuberculosis, Enterococcus faecium, Enterobacter spp, and *Streptococcus* Group B as the most threatening pathogens due to the prevalence of multidrug-resistant (MDR) strains. Bacteria have an outstanding genetic plasticity that allows them to respond to a wide array of hazards, including the presence of antibiotics. The MDR microorganisms employ various mechanisms to resist numerous antibiotics, including inactivation of the active compound, reducing intracellular antibiotic concentration by limiting the influx or overexpressing efflux pumps, and modifying or bypassing the antibiotic´s target site [5–8]. Enzymatic mechanisms against β-lactam antibiotics, such as Extended-Spectrum and Metallo-β-lactamases enzymes (ESBL and MBLs, respectively), can be coded in Gram-negative species [9,10]. Similarly, Gram-positive bacteria have developed mechanisms to alter penicillin-binding proteins (PBPs) through successive mutations in *mec* genes and other non-classical mechanisms [11]. As one of the most widespread examples of antibiotic resistance in Gram-positive pathogens, methicillin-resistant *Staphylococcus aureus* (MRSA) clones acquire a chromosomal cassette (SCC*mec*) and plasmid-mediated genes such as *blaZ* or *vanA*, making infections by this bacterium difficult to treat [12–14].

Addressing AMR requires a One Health approach integrating human, animal, and environmental health. The One Health Quadripartite (FAO, WOAH, WHO, UNEP) coordinates actions to ensure affordable

diagnostics across this spectrum. Research in microbiology, epidemiology, and social sciences is especially critical in low- and middle-income countries (LMICs) [15-18]. Moreover, the WHO research agenda for AMR in human health has already identified 40 research priorities to be addressed by the year 2030, including developing rapid, point-of-care diagnostics to distinguish bacterial, viral, and non-infectious conditions, particularly in low-resource settings [19-20]. However, funding for such diagnostics remains scarce, even compared to antibiotics [3].

AMR also burdens global economies and health systems, disproportionately affecting LMICs where poverty, poor hygiene, limited diagnostics, and restricted access to treatment increase disease burden [18,21]. In this context, advances in molecular techniques (e.g., GeneXpert MTB/RIF, MLST, PFGE, PCR-REP) enhance AMR tracking, but they usually detect only specific mutations [20,22-24]. Thus, culture and MIC testing remain the gold standard in LMICs, despite requiring weeks to produce results for pathogens like *M. tuberculosis* and *K. pneumoniae* [25,26]. By contrast, molecular probes such as those used in real-time PCR enable rapid detection of resistance genes within hours, supporting timely treatment and infection control.

This study aimed to identify MDR pathogens in clinical samples from a tertiary hospital in Ecuador, assess their phenotypic antibiotic susceptibility, and compare results with genotypic resistance (particularly carbapenemases) using TaqMan probes. By correlating

both approaches, we sought to highlight efficient, accurate diagnostic tools for MDR pathogens in resource-limited settings.

Patients and methods

Sample collection and processing

Clinical samples were collected from patients admitted to a tertiary care hospital in Ecuador, between September 2022 and September 2023. Samples came from various sources, including urine, blood cultures, rectal swabs, catheter samples (thigh fistula, bladder catheter), bronchial secretions, and sputum. All samples were collected at the microbiology laboratory of the hospital.

For this study, only bacterial isolates displaying a resistant phenotype—primarily to carbapenem antibiotics—were selected for further analysis. Selection was performed by the microbiology laboratory based on the results of routine antimicrobial susceptibility testing. No clinical or epidemiological data from the patients were available, as the main objective was to characterize resistance patterns and associated resistance genes in this subset of resistant isolates.

The samples were inoculated onto routine culture media based on their origin: 5% sheep blood agar and MacConkey agar for general bacterial isolation, and ChromAgar Orientation (bioMérieux) for urine samples. The cultures were processed according to standard laboratory protocols.

Bacterial identification and antimicrobial susceptibility

Bacterial identification and antimicrobial susceptibility were determined using the VITEK-2 automated system (BioMérieux, Inc., Durham, NC, USA) for 19 antimicrobial agents. Antibiotics tested included: Amikacin (AMK), Ampicillin-Sulbactam (SAM), Cefazolin (CFZ), Cefepime (FEP), Ceftazidime (CAZ), Ceftolozane-Tazobactam (C/T), Ceftriaxone (CRO), Ciprofloxacin (CIP), Ertapenem (ETP), Fosfomycin (FOF), Gentamicin (GEN), Imipenem (IMP), Meropenem (MEM), Nitrofurantoin (NIT), Norfloxacin (NOR), Piperacillin-Tazobactam (TZP), Tazobactam (TAZ), Tigecycline (TGC), and Trimethoprim-Sulfamethoxazole (SXT). As aforementioned, identification and susceptibility testing were performed using group-specific VITEK-2 cards; therefore, not all antibiotics were tested or clinically interpretable for all isolates, particularly in the presence of known intrinsic resistance. Susceptibility patterns were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2023 guidelines.

Genomic DNA Extraction and Detection of Carbapenemase and ESBL

Genomic DNA from bacterial isolates was extracted using the Maelstrom 4800 (TANBead, Taoyuan, Taiwan) and KingFisher Flex (Thermo Fisher Scientific, Waltham, USA) automated systems, following the manufacturers' instructions. The quality and concentration of the extracted DNA were assessed using the Qubit 4 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA).

Resistance genes *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP-1}$, *bla*$_{OXA-48}$, and *bla*$_{CTX-M}$ were detected using multiplex PCR with specific validated TaqMan

probes (Supplementary Table S1) in a QuantStudio 5 real-time PCR system of Applied Biosystems (ThermoFisher, Waltham, MA, USA). To optimize detection, the reactions were divided into two multiplex mixtures: the first included probes for *bla*$_{KPC}$, *bla*$_{NDM}$, and *bla*$_{VIM}$ genes, along with the human internal control C-Human; the second contained probes for *bla*$_{IMP-1}$, *bla*$_{OXA-48}$, and *bla*$_{CTX-M}$ genes, also with the human internal control C-Human. Each PCR run included no-template controls (NTC) to monitor potential contamination. Positive controls consisted of DNA from phenotypically carbapenem-resistant clinical isolates, identified using the VITEK-2 automated system and subsequently confirmed to carry the target genes by TaqMan assays. These isolates, maintained as part of the laboratory's reference collection, were used to ensure consistent amplification performance across all assays.

Each PCR reaction contained a final volume of 16 µL, consisting of 1 µL of multiplex mix, 6 µL of TaqPath enzyme (Thermo Fisher Scientific, Waltham, MA, USA), and 9 µL of extracted DNA. The amplification protocol consisted of an initial denaturation step at 25°C for 2 minutes, followed by 2 minutes at 95°C, and 40 amplification cycles: denaturation at 95°C for 0.3 seconds and annealing/extension at 60°C for 30 seconds.

The PCR results were analyzed using QuantStudio 5 software, allowing for the determination of the presence of the evaluated resistance genes.

Data Analysis of Bacterial Isolates

For the analysis of data obtained from bacterial isolates, various bioinformatics tools and R libraries were employed to evaluate phenotypic antibiotic resistance, the distribution of resistance genes, and the relationship between microorganisms, resistance profiles, and genes.

First, antibiotic sensitivity was analyzed by evaluating the phenotypic distribution of resistance to different antibiotics. Data processing was performed using the R packages (libraries) dplyr for data manipulation and grouping, tidyr for transforming data into long format, and ggplot2 for creating stacked bar charts that represented proportions of resistance, susceptibility, and intermediates. Additionally, scales package was used to format the chart axes into percentages. Subsequently, the distribution of carbapenemase resistance genes (*bla*$_{\text{KPC}}$, *bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$, *bla*$_{\text{OXA-48}}$, and *bla*$_{\text{IMP-1}}$) was analyzed. This analysis included grouping and counting the presence or absence of genes using dplyr and representing gene intersections through Venn diagrams generated with ggvenn. Furthermore, the diversity of microorganisms by isolation site was evaluated using dplyr to calculate frequencies, ggplot2 to generate stacked bar charts and pie charts, and forcats to reorder factors on the chart axes. To differentiate species in visualizations, color palettes from RColorBrewer were used, while patchwork allowed the combination of charts into composite figures.

Additionally, a Multiple Factor Analysis (MFA) was conducted to explore the joint relationship among microorganisms, phenotypic

susceptibility, and presence of resistance genes. This analysis was performed using FactoMineR package for MFA execution and factoextra for visualizing individuals, groups, and variables. Finally, hierarchical clustering analysis and heatmaps were performed to identify similarities in resistance profiles and genes from the isolates. The Gower distance was calculated using the daisy function from the cluster package. Heatmaps were generated with pheatmap, incorporating cluster annotations.

Results

Distribution of Clinical Samples and Bacterial Isolates

A total of 216 clinical samples were collected from various sources (Figure 1), with urine being the most common (80/216, 37.0%), followed by soft tissue secretions (38/216, 17.6%), blood (37/216, 17.1%), and tracheal secretions (17/216, 7.9%). Other sample types, such as bronchial secretions (13/216, 6.0%), sputum (8/216, 3.7%), rectal swabs (7/216, 3.2%), and bronchoalveolar lavage (4/216, 1.9%), were collected less frequently.

Klebsiella pneumoniae was the predominant bacterial species, representing 59.7% of total isolates, followed by *Pseudomonas aeruginosa* (11.6 %), *Serratia marcescens* (8.3 %), *Acinetobacter baumannii* (6.9%), and *Escherichia coli* (6.5%) (Figure 1). Other identified species included in descending order: *Enterobacter cloacae*, *Providencia stuartii*, *Morganella morganii*, *Proteus mirabilis*, *Aeromonas hydrophila*, *Enterobacter hormaechei*, *Klebsiella*

aerogenes, *K. oxytoca*, and *Stenotrophomonas maltophilia*, though at lower frequencies.

Antimicrobial Susceptibility Profiles

Antimicrobial susceptibility analysis revealed high levels of resistance among the bacterial isolates. Carbapenems demonstrated significant resistance, with rates of 100.0% for imipenem, 96.9% for ertapenem, and 95.7% for meropenem. Similarly, third-generation cephalosporins also showed high resistance, with ceftriaxone at 99.3% and ceftazidime at 92.0% as the most prevalent. On the other hand, ciprofloxacin resistance among fluoroquinolones reached 88.7%. Finally, among aminoglycosides, gentamicin exhibited 66.5% resistance, while amikacin showed a lower resistance rate of 27.5%, indicating better performance. In contrast, tigecycline demonstrated the highest susceptibility, with 80.8% of isolates susceptible (Figure 2).

Detection of Carbapenemase Genes

Molecular analysis using multiplex PCR identified carbapenemase genes in the bacterial isolates. Among the 216 isolates evaluated, the bla_{KPC} gene was the most frequently detected, present in 70.8% (n=153). In smaller proportions, the bla_{NDM} and bla_{VIM} genes were identified, with prevalences of 9.7% (n=21) and 2.8% (n=6), respectively (Figure 3). On the other hand, the $bla_{\text{OXA-48}}$ gene was detected in a single isolate, representing 0.5% (n=1) of the analyzed population. A small number of isolates (n=10) exhibited multiple carbapenemase genes (Supplementary Table S2). The most common

combinations included the coexistence of *bla*$_{KPC}$ and *bla*$_{NDM}$ genes (n=4), followed by *bla*$_{KPC}$ and *bla*$_{VIM}$ genes (n=5), whereas there was a single case for *bla*$_{KPC}$ and *bla*$_{OXA-48}$ genes (n=1) (Figure 3). Neither *bla*$_{IMP-1}$ nor *bla*$_{CTX-M}$ was detected in any of the evaluated isolates. These isolates showed uniform resistance to carbapenems and to third- and fourth-generation cephalosporins, aligned with the presence of multiple carbapenemase genes. However, resistance to non-β-lactam antibiotics was not consistent: amikacin susceptibility varied among isolates, and tigecycline remained active in several cases. Thus, these findings suggest that carbapenemase co-production does not necessarily result in a broader resistance profile compared with single-gene producers, at least when using categorical S/I/R interpretations.

Phylogenetic Analysis and Antimicrobial Susceptibility Profiles of Clinical Bacterial Isolates

The joint analysis of genotypic and phenotypic profiles, represented in the dendrogram and heat map in Figure 4, showed a marked concordance between the presence of carbapenemase genes and resistance to carbapenems and cephalosporins in most clinical isolates. For example, 89.5% of *bla*$_{KPC}$-positive isolates were resistant to ertapenem and 97.4% to meropenem, while resistance to ceftriaxone and ceftazidime reached 85% and 90.2 %, respectively. This trend was especially notable in *K. pneumoniae*, the most represented species (129 isolates), where 94.6% were positive for at least one carbapenemase gene and presented 87.4% overall

resistance to carbapenems and cephalosporins. Similarly, *S. marcescens* (18 isolates) and *E. coli* (14 isolates) showed 94.4% and 78.6% positivity for carbapenemase genes, respectively, and were universally resistant to carbapenems and cephalosporins.

However, Figure 4 also reveals isolates with phenotypic carbapenem resistance in the absence of detectable carbapenemase genes. For instance, all isolates of *P. aeruginosa* showed phenotypic resistance to carbapenems and cephalosporins, but only 32 % (n=25) of them were positive for any of the resistance genes analyzed. Thus, isolates EC-174, EC-175, EC-180, EC-184, EC-185, and EC-188 presented resistance to carbapenems and other β-lactams (although some retained susceptibility to ceftazidime or cefepime) regardless of the genetic profile. On the other hand, in *A. baumannii* (15 isolates), only three were positive for carbapenemase genes, but most showed an MDR phenotype. This is exemplified by isolates EC-001, EC-003, EC-004, EC-006, EC-007, and EC-008, which exhibited resistance to carbapenems, cephalosporins, and fluoroquinolones, although intermediate susceptibility to certain antibiotics was observed in some cases. Similarly, *S. maltophilia* EC-215 was resistant to multiple drugs, including piperacillin/tazobactam and carbapenems, whereas it was only susceptible to gentamicin, ceftazidime, and trimethoprim/sulfamethoxazole. *M. morganii* EC-165 and EC-166 showed resistance to fluoroquinolones and sulfonamides, with partial susceptibility to meropenem and amikacin. Overall, Figure 4 not only illustrates the genetic diversity of clinical isolates using the

hierarchical dendrogram but also visualizes the high prevalence of resistance (in red) to carbapenems, third-generation cephalosporins, and fluoroquinolones, especially in *K. pneumoniae* and *A. baumannii*. Although the correlation between the presence of carbapenemase genes and phenotypic resistance is high, the detection of resistant isolates without identified genes highlights the importance of continuing to investigate alternative resistance mechanisms in this clinical setting, as will be discussed below.

Discussion

The results of this study highlight the worrying prevalence of AMR in clinical isolates obtained from a tertiary hospital in Ecuador. We found high resistance to carbapenems (largely driven by carbapenemase genes such as bla_{KPC}), third-generation cephalosporins, and fluoroquinolones. These findings emphasize the urgent need for strategies to curb the spread of MDR bacteria.

In our study, phenotypic analysis by MIC testing showed extremely high resistance to crucial antibiotics: >95% for carbapenems (ertapenem, meropenem) and >85% for third- and fourth-generation cephalosporins (ceftriaxone, ceftazidime, and cefepime), with minimal residual susceptibility (only 0.7% and 2.6% of susceptible strains for each generation, respectively). This pattern aligns with regional and global studies connecting carbapenemase dissemination to broad resistance [27–32].

Aminoglycoside resistance was lower but relevant. Amikacin retained higher susceptibility (>50%) than gentamicin (26.6%), consistent with other Latin American and Asian studies [29,33]. Thus, over half of the isolates evaluated were susceptible to amikacin, whereas gentamicin susceptibility was lower (26.6%). These results suggest that amikacin could be a therapeutic alternative, although the presence of intermediate resistance requires cautious use [34]. Furthermore, high resistance to gentamicin limits its effectiveness in MDR strains, probably due to the indiscriminate use of aminoglycosides in some regions [27,30,35]. Conversely, tigecycline showed high susceptibility (80.8%) against MDR bacteria, a result similar to that reported by other authors in Ecuador [36]. This supports its role in settings where carbapenems and cephalosporins fail, although its use should depend on the infection site and drug pharmacokinetics [37,38].

Genotypically, multiplex PCR identified the *bla*$_{\text{KPC}}$ gene as the predominant carbapenemase one (70.8% of isolates), followed by the *bla*$_{\text{NDM}}$ (9.7%), *bla*$_{\text{VIM}}$ (2.8%), and *bla*$_{\text{OXA-48}}$ (0.5%). These results are consistent with those reported by Soria-Segarra et al. (2024) in Ecuador, as well as in other regions such as China, where the *bla*$_{\text{KPC}}$ gene was identified as the main resistance mechanism in *K. pneumoniae* isolates, followed by the *bla*$_{\text{NDM}}$, *bla*$_{\text{VIM}}$, and *bla*$_{\text{OXA-48}}$ genes [30,34,36,39]. In contrast, Joshi et al. (2023) found a predominance of *bla*$_{\text{OXA-48}}$ (84.5%) and *bla*$_{\text{NDM}}$ (58.6%) in southern India [40]. Similarly, Giri et al. (2021) reported that most isolates from West-central India carried *bla*$_{\text{NDM}}$ and *bla*$_{\text{OXA-48}}$ genes, with a

combination of these as the main resistance mechanism, especially in *K. pneumoniae* [41]. In contrast, the *bla*$_{\text{IMP-1}}$ gene has been reported in Ecuador [42], but its absence in our clinical samples, as well as in those from other recent studies in the country, may indicate limited regional circulation or differences in methodological sensitivity [36]. Similarly, despite the very high ceftriaxone resistance rate observed (99.3%), *bla*$_{\text{CTX-M}}$ was not detected. This discordance may reflect the presence of alternative ESBL families or non-CTX-M resistance mechanisms in our cohort.

Although our work focuses on clinical isolates, it is important to consider these findings within a One Health context. Thus, carbapenemase genes, including OXA-type oxacillinases, have also been reported in *Acinetobacter* isolates from highly polluted urban rivers in Quito [43]. This indicates that clinically relevant carbapenem resistance determinants, such as *bla*$_{\text{KPC}}$ and *bla*$_{\text{OXA-48,}}$ detected in our isolates may not be restricted to hospital settings but are also present in environmental settings. Although our study focuses on clinical samples, these environmental findings suggest the existence of a broader resistome that may facilitate the regional circulation of carbapenemase genes.

Co-occurrence of multiple carbapenemase genes (*bla*$_{\text{KPC}}$+*bla*$_{\text{NDM}}$, *bla*$_{\text{KPC}}$+*bla*$_{\text{VIM}}$, *bla*$_{\text{KPC}}$+*bla*$_{\text{OXA-48}}$) complicates treatment. The co-harboring of *bla*$_{\text{KPC}}$ and *bla*$_{\text{NDM}}$, reported in several countries in Latin America and Asia [31,40,41,44], further exacerbates resistance to β-lactam antibiotics and severely limits available therapeutic options.

Argentina, Colombia, Uruguay, Brazil, and Ecuador have already informed the simultaneous detection of these genes in *K. pneumoniae*, proving the spread of these resistance mechanisms and the consequent difficulties for diagnosis and treatment [31,44,45]. On the other hand, the combination of *bla*$_{KPC}$ and *bla*$_{VIM}$ genes, less frequent in our study, has usually been linked to hospital outbreaks and greater clonal dissemination [44]. In addition, the coexistence of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ is especially worrying due to its impact on therapeutic options and the possibility of horizontal transfer of resistance genes [46,47]. In Ecuador, although the simultaneous harboring of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ in *E. coli* isolates was reported in 2021 and 2023 [45,48], to the best of our knowledge, this combination has not been documented in *K. pneumoniae* to date. In this work, we report for the first time in Ecuador the coexistence of the *bla*$_{KPC}$ and *bla*$_{OXA-48}$ genes in a *K. pneumoniae* isolate, a finding of great epidemiological and clinical relevance. The coexistence of *bla*$_{KPC}$ and *bla*$_{OXA-48}$ genes may have important clinical implications, since OXA-48-like enzymes can evolve under ceftazidime-avibactam pressure toward reduced avibactam susceptibility and enhanced ceftazidime hydrolysis, even if this evolution comes with certain biological trade-offs [49,50]. Since clinical evidence shows that ceftazidime-avibactam remains one of the most effective options available -offering significantly lower mortality rates than other regimens for infections caused by KPC- or OXA-48-producing *Enterobacterales*- [51], the emergence of this combination of carbapenemases could jeopardize one of the few

remaining therapeutic alternatives. Given the importance of this species as an agent of nosocomial infections and its ability to spread resistance, the identification of this genetic profile underscores the need to strengthen molecular surveillance and infection control programs in the country.

K. pneumoniae was the most frequently isolated species (59.7 %), representing 94.6 % of isolates positive for at least one carbapenemase gene. This finding is consistent with recent national studies, confirming the role of this species as a major reservoir and driver of resistance dissemination across Ecuadorian hospitals [36,42]. This high prevalence of resistance to carbapenems, often accompanied by cephalosporin resistance, also aligns with international literature identifying *K. pneumoniae* as a leading cause of MDR nosocomial infections, as aforementioned, and underscores its extensively resistant profile and a pivotal role in the national and international spread of resistance mechanisms [30,40,42,52].

In turn, *P. aeruginosa* was the second most frequently isolated species. Although only 32 % of isolates presented carbapenemase genes, widespread phenotypic resistance to both carbapenems and cephalosporins was observed. This suggests the possible involvement of other resistance mechanisms, such as the presence of less common genes (*bla*$_{SPM}$, *bla*$_{GIM}$, *bla*$_{SIM}$, and *bla*$_{GES}$) [53], as well as intrinsic mechanisms, including the lack of the porin OprD (conferring imipenem resistance) and the overexpression of efflux pumps, especially MexAB-OprM (conferring meropenem resistance) [54].

These factors also contribute significantly to the observed resistance and should be considered when designing molecular detection panels. At the same time, because these mechanisms fall outside the scope of our carbapenemase-focused PCR assays, the negative genotypic results in our isolates could be expected and might not reflect true genotype–phenotype discrepancies. In contrast, among the 15 *A. baumannii* isolates, only three were positive for the tested carbapenemase genes, although most showed elevated MIC values for meropenem. This finding also points to the potential involvement of other resistance mechanisms, including the expression of other OXA-type carbapenemase genes (*bla*$_{\text{OXA-23}}$, *bla*$_{\text{OXA-24}}$, *bla*$_{\text{OXA-40}}$, and *bla*$_{\text{OXA-58}}$) and the overexpression of the intrinsic carbapenemase *bla*$_{\text{OXA-51}}$, both established as key contributors to carbapenem resistance in this species [55–57]. Among these acquired OXA-type carbapenemases, *bla*$_{\text{OXA-23}}$ has consistently been reported as the most prevalent in *A. baumannii* across Latin America, as evidenced by prior and recent regional surveillance data [58-60].

In contrast, a different scenario was observed in less common isolates such as *Enterobacter cloacae*, *E. hormaechei*, *Klebsiella aerogenes*, *K. oxytoca*, *Proteus mirabilis*, and *Providencia stuartii*, where a clear concordance was found between the presence of carbapenemase genes and phenotypic resistance to multiple antibiotics, confirming their MDR status.

The observed genetic heterogeneity demonstrates bacterial adaptability and highlights the need for improved diagnostics. While

real-time PCR and next-generation sequencing (NGS) enhance detection, our panel was limited to selected genes, potentially overlooking other mechanisms. Broader surveillance, including the use of whole genome sequencing (WGS), is essential to detect emerging resistance and inform prevention. Combining phenotypic and molecular methods remains indispensable for guiding therapy and controlling MDR infections [61–66].

This study has several limitations. Firstly, the absence of clinical and demographic information on the patients limits the ability to correlate microbiological findings with risk factors, clinical outcomes, or epidemiological characteristics of the population. Secondly, molecular analysis was limited to a specific panel of resistance genes, possibly overlooking other mechanisms, as we have previously pointed out. Finally, being a single-center study, the findings may not be generalizable to other different settings.

Conclusions

This study demonstrates the severity of AMR in Ecuadorian hospitals, driven by high carbapenemase prevalence (mainly *bla*$_{\text{KPC}}$) and widespread multidrug resistance. The novel report of *bla*$_{\text{KPC}}$/*bla*$_{\text{OXA-48}}$ genes coexistence in *K. pneumoniae* underscores the urgency of strengthening molecular surveillance, optimizing antimicrobial use, and reinforcing infection control. Future studies using WGS would be essential to achieve a more comprehensive epidemiological

understanding and to support the development of rapid, locally tailored diagnostic tests.

List of abbreviations

AMK: Amikacin

AMR: Antimicrobial resistance

C/T: Ceftolozane-Tazobactam

CAZ: Ceftazidime

CDC: American Centers for Disease Control and Prevention

CFZ: Cefazolin

CIP: Ciprofloxacin

CLSI: Clinical and Laboratory Standards Institute

CRO: Ceftriaxone

ESBL: Extended-Spectrum enzyme

ETP: Ertapenem

FOF: Fosfomycin

GEN: Gentamicin

FEP: Cefepime

IMP: Imipenem

MBLs: Metallo-β-lactamases enzymes

MDR: multidrug-resistant

MEM: Meropenem

PBPs: Penicillin-binding proteins

LMICs: Low- and middle-income countries

MFA: Multiple Factor Analysis

MRSA: methicillin-resistant *Staphylococcus aureus*

SAM: Ampicillin-Sulbactam

NGS: next-generation sequencing

NIT: Nitrofurantoin

NOR: Norfloxacin

NTC: No-template controls

TZP: Piperacillin-Tazobactam

TAZ: Tazobactam

TGC: Tigecycline

SXT: Trimethoprim-Sulfamethoxazole

WGS: whole genome sequencing

WHO: The World Health Organization

Declarations

Ethical approval

The study was approved by the Ethics Committee of the University Espíritu Santo (code 2022-001A), certified by the Ministry of Public Health of Ecuador, and conducted in accordance with the principles outlined in the Declaration of Helsinki.

The requirement to obtain informed consent was waived by the same Ethics Committee (Ethics Committee of the Universidad Espíritu Santo) because the study included only isolates from a collection, the data were anonymized, and no patient information was disclosed.

Consent for publication

Not Applicable

Competing interests

The authors declare no conflicts of interest.

Author´s contribution and potential conflicts of interest

DFS carried out the experiments, gathered clinical information, and wrote the manuscript with support from CMGD and JCFC; JCFC and GML conceived the original idea; CMGD and JCFC supervised the project.

Availability of data and materials

The datasets generated and analysed during the current study are available from David Fernández-Salvatierra (University of Malaga) and Juan Carlos Fernández-Cadena (Espíritu Santo University; Harvard Medical School and Brigham and Women´s Hospital) upon request, while maintaining the necessary confidentiality for sensitive data. Thus, access requests should be directed to fernandezsalvatierra@uma.es and juan.fernandez@um6p.ma, respectively.

Funding

Funding for open access charges was provided by the Ecotec University.

References

1. Murray CJL, Ikuta KS, Sharara F, Swetschinski L, Robles Aguilar G, Gray A, *et al.* Global burden of bacterial antimicrobial resistance in 2019: a systematic analysis. *The Lancet* 2022; 399: 629-55.

2. Naghavi M, Vollset SE, Ikuta KS, Swetschinski LR, Gray AP, Wool EE, *et al*. Global burden of bacterial antimicrobial resistance

1990–2021: a systematic analysis with forecasts to 2050. *The Lancet* 2024; 404: 1199-226.

3. The Lancet. Antimicrobial resistance: an agenda for all. *The Lancet* 2024; 403: 2349.

4. Woolhouse MEJ. One Health approaches to tackling antimicrobial resistance. Science in One Health 2024; 3: 100082. https://linkinghub.elsevier.com/retrieve/pii/S2949704324000210

5. Gaurav A, Bakht P, Saini M, Pandey S, Pathania R. Role of bacterial efflux pumps in antibiotic resistance, virulence, and strategies to discover novel efflux pump inhibitors. *Microbiology* 2023; 169: 0.001333.

6. Vivekanandan KE, Kumar PV, Jaysree RC, Rajeshwari T. Exploring molecular mechanisms of drug resistance in bacteria and progressions in CRISPR/Cas9-based genome expurgation solutions. *Glob Med Genet* 2025; 12: 100042.

7. Hajiagha MN, Kafil HS. Efflux pumps and microbial biofilm formation. *Infect Genet Evol* 2023; 112: 105459.

8. Darby EM, Trampari E, Siasat P, Gaya MS, Alav I, Webber MA, *et al.* Molecular mechanisms of antibiotic resistance revisited. *Nat Rev Microbiol* 2023; 21: 280-95.

9. Castanheira M, Simner PJ, Bradford PA. Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC Antimicrob Resist* 2021; 3: dlab092.

10. Husna A, Rahman MdM, Badruzzaman ATM, Sikder MH, Islam MR, Rahman MdT, *et al*. Extended-Spectrum β-Lactamases (ESBL): Challenges and Opportunities. *Biomedicines* 2023; 11: 2937.

11. Lai LY, Satishkumar N, Cardozo S, Hemmadi V, Marques LB, Huang L, *et al*. Altered PBP4 and GdpP functions synergistically mediate MRSA-like high-level, broad-spectrum β-lactam resistance in *Staphylococcus aureus*. *mBio* 2024; 15: e02889-23.

12. Ghimire L, Banjara MR, Abdulla AM. Antibiotic Susceptibility of *Staphylococcus Aureus* with *van*A and *mec*A Genes. *J Nepal Health Res Counc* 2024; 21: 616-22.

13. Maharjan M, Sah AK, Pyakurel S, Thapa S, Maharjan S, Adhikari N, *et al*. Molecular Confirmation of Vancomycin-Resistant *Staphylococcus aureus* with *van*A Gene from a Hospital in Kathmandu. *Int. J. Microbiol* 2021; 2021:1-8.

14. Smith JT, Eckhardt EM, Hansel NB, Rahmani Eliato T, Martin IW, Andam CP. Genome Evolution of Invasive Methicillin-Resistant *Staphylococcus aureus* in the Americas. *Microbiol Spectr*; 10: e00201-22.

15. Pitt SJ, Gunn A. The One Health Concept. *Br J Biomed Sci* 2024; 81:12366.

16. Centers for Disease Control and Prevention (CDC). Antimicrobial Resistance: Causes and How It Spreads [Internet]. CDC; 2024. https://www.cdc.gov/antimicrobial-resistance/causes/index.html#:~:text=Antimicrobial%2Dresistant%2

0germs%20can%20share,or%20even%20impossible%2C%20to%20treat

17. The Food and Agriculture Organization of the United Nations (FAO), the United Nations Environment Programme (UNEP), the World Organisation for Animal Health (WOAH, founded as OIE), and the World Health Organization (WHO). One Health Joint Plan of Action (2022–2026): Working Together for the Health of Humans, Animals, Plants and the Environment. 1st ed. Geneva: World Health Organization; 2022. 1 p.

18. Rousham EK, Unicomb L, Islam MA. Human, animal and environmental contributors to antibiotic resistance in low-resource settings: integrating behavioural, epidemiological and One Health approaches. *Proc Biol Sci* 2018; 285: 20180332.

19. Bertagnolio S, Dobreva Z, Centner CM, Olaru ID, Donà D, Burzo S, *et al*. WHO global research priorities for antimicrobial resistance in human health. *The Lancet Microbe* 2024; 5: 100902.

20. McAdams D, Wollein Waldetoft K, Tedijanto C, Lipsitch M, Brown SP. Resistance diagnostics as a public health tool to combat antibiotic resistance: A model-based evaluation. *PLoS Biol* 2019; 17: e3000250.

21. Iskandar K, Molinier L, Hallit S, Sartelli M, Catena F, Coccolini F, *et al*. Drivers of Antibiotic Resistance Transmission in Low- and Middle-Income Countries from a “One Health” Perspective-A Review. *Antibiotics* 2020; 9: 372.

22. Hussain S, Sultan S, Riaz S, Hussain H, Javed H, Mazhar R. Synergy of Xpert (MTB/RIF) and Line probe assay for detection of rifampicin-resistant strains of *Mycobacterium tuberculosis*. *J Infect Dev Ctries* 2024; 18: 1241-8.

23. Hernández-Alomía F, Bastidas-Caldes C, Ballesteros I, Tenea GN, Jarrín-V. P, Molina CA, *et al*. Beta-Lactam Antibiotic Resistance Genes in the Microbiome of the Public Transport System of Quito, Ecuador. *IJERPH* 2023; 20: 1900.

24. Vatterrodt D, Lee J, Ho D, Stevig C, Chow SK. Misidentification and misreporting of antibiotic resistance in *Kluyvera* bacteremia by blood culture molecular identification panels. Microbiol Spectr 2024; 12: e00542-24.

25. Palavecino EL. Rapid Methods for Detection of MRSA in Clinical Specimens. *Methods Mol Biol* 2020: 2069:29-45.

26. Shahmohammadi MR, Nahaei MR, Akbarzadeh A, Milani M. Clinical test to detect *mec*A and antibiotic resistance in *Staphylococcus aureus*, based on novel biotechnological methods. *Artif Cells Nanomed Biotechnol* 2016; 44: 1464-8.

27. Ortega D, Alcocer I, Zurita J. Alta prevalencia de enzimas modificadoras de aminoglucósidos (EMAs) en aislados clínicos de *Klebsiella pneumoniae* (KPN) productores de β-lactamasas de espectro extendido (BLEE). *REMCB* 2009; 30: 16-27.

28. Özyazici G, Özkaya E, Kaya H, Kocatürk Sel S, Altinbaşak H, Başari F, *et al*. Investigation of *bla*KPC Gene by PCR in Carbapenem-

Resistant *Escherichia coli* and *Klebsiella pneumoniae* Clinical İsolates in a Tertiary Care Hospital in Turkey. *MJIMA* 2019; 8: 3.

29. Firmo EF, Cabral AB, Oliveira ÉM de, Lopes ACS. Emergence of *aph*(3')-VI and accumulation of aminoglycoside-modifying enzyme genes in KPC-2-possessing *Enterobacter aerogenes* isolates from infections and colonization in patients from Recife-PE, Brazil. *Rev Soc Bras Med Trop* 2019; 52: e20180460.

30. Han R, Shi Q, Wu S, Yin D, Peng M, Dong D, *et al*. Dissemination of Carbapenemases (KPC, NDM, OXA-48, IMP, and VIM) Among Carbapenem-Resistant *Enterobacteriaceae* Isolated from Adult and Children Patients in China. *Front Cell Infect Microbiol* 2020; 10: 314.

31. García-Betancur JC, Appel TM, Esparza G, Gales AC, Levy-Hara G, Cornistein W, *et al*. Update on the epidemiology of carbapenemases in Latin America and the Caribbean. *Expert Rev Anti Infect Ther* 2021; 19: 197-213.

32. Rodríguez-Pérez AU, Madruga-Fernández M de la C, Hernández-Sánchez MC. *Enterobacterales* that produce betalactamases in clinical isolations of hospitalized patients inside an institution of secondary medical level. Havana - CUBA, 2021. *REM HRL* 2022; 8(3).

33. Abhishek S. Genotypic Distribution and Antimicrobial Susceptibilities of Carbapenemase-Producing *Enterobacteriaceae* Isolated in Tertiary Care Hospital in South India. *J Pure Appl Microbiol* 2022;16: 2488-2495.

34. Cui X, Zhang H, Du H. Carbapenemases in Enterobacteriaceae: Detection and Antimicrobial Therapy. *Front Microbiol* 2019; 10.

35. Díaz Q P, Bello T H, Domínguez Y M, Trabal F N, Mella M S, Zemelman Z R, *et al*. Resistance to gentamicin, amikacin and ciprofloxacin among nosocomial isolates of *Klebsiella pneumoniae* subspecie *pneumoniae* producing extended spectrum ß-lactamases. *Rev Méd Chile* 2004; 132: 1173-8.

36. Soria-Segarra C, Soria-Segarra C, Molina-Matute M, Agreda-Orellana I, Núñez-Quezada T, Cevallos-Apolo K, *et al*. Molecular epidemiology of carbapenem-resistant gram-negative bacilli in Ecuador. *BMC Infect Dis* 2024; 24: 378.

37. Ramirez J, Dartois N, Gandjini H, Yan JL, Korth-Bradley J, McGovern PC. Randomized Phase 2 Trial To Evaluate the Clinical Efficacy of Two High-Dosage Tigecycline Regimens *versus* Imipenem-Cilastatin for Treatment of Hospital-Acquired Pneumonia. *Antimicrob Agents Chemother* 2013; 57: 1756-62.

38. Zha L, Pan L, Guo J, French N, Villanueva EV, Tefsen B. Effectiveness and Safety of High Dose Tigecycline for the Treatment of Severe Infections: A Systematic Review and Meta-Analysis. *Adv Ther* 2020; 37: 1049-64.

39. Li C, Chen R, Qiao J, Ge H, Fang L, Liu R, *et al*. Distribution and molecular characterization of carbapenemase-producing gram-negative bacteria in Henan, China. *Sci Rep* 2024; 14: 14418.

40. Joshi DN, Shenoy B, Mv B, Adhikary R, Shamarao S, Mahalingam A. Prevalence of Carbapenem-Resistant *Enterobacteriaceae* and the

Genes Responsible for Carbapenemase Production in a Tertiary Care Hospital in South India. *EMJ* 2023. https://doi.org/10.33590/emj/10300425

41. Giri S, Karade S, Sen S. Genotypic characterization of carbapenem resistant Enterobacterales in clinical isolates from western Maharashtra. *Indian J Med Microbiol* 2021; 39: 500-3.

42. National Institute of Public Health Research. Surveillance of ESBL and Carbapenemases in Ecuador, 2018–2021 period. INSPI; 2022 https://www.investigacionsalud.gob.ec/webs/ram/wp-content/uploads/2022/11/2do-Boletin-CRN-Resistencia-Antimicrobiana.pdf

43. Sotomayor N, Villacis JE, Burneo N, Reyes J, Zapata S, Bayas-Rea RLÁ. Carbapenemase genes in clinical and environmental isolates of *Acinetobacter* spp. from Quito, Ecuador. *PeerJ*. 2024; 12:e17199.

44. Guerra-Sarmiento M, Ruíz-Martin-Leyes F, Arzuza-Ortega L, Maestre-Serrano R, Guerra-Sarmiento M, Ruíz-Martin-Leyes F, *et al*. Characterization of multiresistant gram-negative bacilli, isolated in patients hospitalized in health institutions in Barranquilla (Colombia). *Rev Chilena Infectol* 2021; 38: 189-96.

45. Pan American Health Organization / World Health Organization. Epidemiological Alert: Emergence and Increase of New Combinations of Carbapenemases in *Enterobacterales* in Latin America and the Caribbean. PAHO /WHO; 2021. https://www.paho.org/sites/default/files/2023-08/2021-octubre-phe-alerta-epi-carbapenamasassp.pdf

46. Wang YC, Tang HL, Liao YC, Chiou CS, Chen YT, Chiang MK, *et al*. Cocarriage of Distinct blaKPC-2 and blaOXA-48 Plasmids in a Single Sequence Type 11 Carbapenem-Resistant *Klebsiella pneumoniae* Isolate. *Antimicrob Agents Chemother* 2019; 63: 10.1128/aac.02282-18.

47. Yang Y, Yang Y, Ahmed MAEGES, Qin M, He R, Wu Y, *et al*. Carriage of distinct blaKPC-2 and blaOXA-48 plasmids in a single ST11 hypervirulent *Klebsiella pneumoniae* isolate in Egypt. *BMC Genomics* 2022; 23: 20.

48. Linares Flores C, Martinez Freres G, Torres Ponce A, Alcívar Murillo S, Bravo Espinoza R, Yépez Anchundia S. Co-production of carbapenemases: "*in vitro*" challenge for microbiology laboratories, "*in vivo*" challenge for physicians. *INSPILIP* 2023. https://www.inspilip.gob.ec/index.php/inspi/article/view/373

49. Fröhlich C, Sørum V, Thomassen AM, Johnsen PJ, Leiros HS, Samuelsen Ø. OXA-48-Mediated Ceftazidime-Avibactam Resistance Is Associated with Evolutionary Trade-Offs. *mSphere.* 2019; 4: e00024-19.

50. Palwe S, Bakthavatchalam YD, Khobragadea K, Kharat AS, Walia K, Veeraraghavan B. In-Vitro Selection of Ceftazidime/Avibactam Resistance in OXA-48-Like-Expressing *Klebsiella pneumoniae*: In-Vitro and In-Vivo Fitness, Genetic Basis and Activities of β-Lactam Plus Novel β-Lactamase Inhibitor or β-Lactam Enhancer Combinations. *Antibiotics (Basel).* 2021; 10: 1318.

51. Aslan AT, Tanriverdi ES, Kaya SY, Dalgan G, Saltoğlu N, Yılmaz E, et al. Comparison of ceftazidime-avibactam with other appropriate antimicrobial therapy for the treatment of OXA-48- or KPC-producing *Enterobacterales* infections in Türkiye: A multi-centre retrospective matched-cohort study. *Int J Antimicrob Agents.* 2026; 67: 107650.

52. Zhang Y, Wang Q, Yin Y, Chen H, Jin L, Gu B, *et al*. Epidemiology of Carbapenem-Resistant *Enterobacteriaceae* Infections: Report from the China CRE Network. *Antimicrob Agents Chemother* 2018; 62: 10.1128/aac.01882-17.

53. Karampatakis T, Antachopoulos C, Tsakris A, Roilides E. Molecular epidemiology of carbapenem-resistant *Pseudomonas aeruginosa* in an endemic area: comparison with global data. *Eur J Clin Microbiol Infect Dis* 2018; 37: 1211-20.

54. Alimoghadam S, Eslami A, Alimoghadam R, Bahrami Mianrood I, Azizmohammad Looha M, Khodadadi S, *et al*. The frequency of AmpC overproduction, OprD downregulation and OprM efflux pump expression in *Pseudomonas aeruginosa*: A comprehensive meta-analysis. *J Glob Antimicrob Resist* 2024; 39: 159-69.

55. Poirel L, Nordmann P. Carbapenem resistance in *Acinetobacter baumannii*: mechanisms and epidemiology. *Clin Microbiol Infect* 2006; 12: 826-36.

56. Seshatri S, Charan J, Tak V, Nag VL, Varthya SB, Ambwani S. Frequency of OXA-Type Carbapenemases among Carbapenem-Resistant *Acinetobacter baumannii* in Clinical Isolates from Adult Intensive Care Unit in India. *J Lab Physicians* 2023.

https://www.thieme-connect.com/products/ejournals/abstract/10.1055/s-0043-1771243

57. Smith CA, Antunes NT, Stewart NK, Toth M, Kumarasiri M, Chang M, *et al*. Structural Basis for Carbapenemase Activity of the OXA-23 β-Lactamase from *Acinetobacter baumannii*. *Chem Biol* 2013; 20: 1107-15.

58. Higgins PG, Dammhayn C, Hackel M, Seifert H. Global spread of carbapenem-resistant Acinetobacter baumannii. *J Antimicrob Chemother*. 2010; 65: 233-8. Erratum in: *J Antimicrob Chemother*. 2010; 65: 1317.

59. Kiffer CRV, Rezende TFT, Costa-Nobre DT, Marinonio ASS, Shiguenaga LH, Kulek DNO, et al. A 7-Year Brazilian National Perspective on Plasmid-Mediated Carbapenem Resistance in *Enterobacterales, Pseudomonas aeruginosa*, and *Acinetobacter baumannii* Complex and the Impact of the Coronavirus Disease 2019 Pandemic on Their Occurrence. *Clin Infect Dis*. 2023; 77(Suppl 1): S29-S37.

60. Pillonetto M, Wink PL, Melano RG, Jiménez-Pearson MA, Melgarejo Touchet NL, Saavedra Rojas SY, *et al*. Carbapenemases producing gram-negative bacteria surveillance in Latin America and the caribbean: a retrospective observational study from 2015 to 2020. *Lancet Reg Health Am.* 2025; 49: 101185.

61. Abou-assy RS, Aly MM, Amasha RH, Jastaniah S, Alammari F, Shamrani M. Carbapenem Resistance Mechanisms, Carbapenemase

Genes Dissemination, and Laboratory Detection Methods: A Review. *IJPRAS* 2023; 12: 123-38.

62. Ellington MJ, Findlay J, Hopkins KL, Meunier D, Alvarez-Buylla A, Horner C, *et al*. Multicentre evaluation of a real-time PCR assay to detect genes encoding clinically relevant carbapenemases in cultured bacteria. *Int J Antimicrob Agents* 2016; 47: 151-4.

63. Djordjevic SP, Jarocki VM, Seemann T, Cummins ML, Watt AE, Drigo B, *et al*. Genomic surveillance for antimicrobial resistance: a One Health perspective. *Nat Rev Genet* 2024; 25: 142-57.

64. Waddington C, Carey ME, Boinett CJ, Higginson E, Veeraraghavan B, Baker S. Exploiting genomics to mitigate the public health impact of antimicrobial resistance. *Genome Med* 2022; 14: 15.

65. Mejía-Limones I, Andrade-Molina D, Morey-León G, Hidalgo-Olmedo JC, Chang-Asinc JG, Fernández-Cadena JC, *et al*. Whole-genome sequencing of *Klebsiella pneumoniae* MDR circulating in a pediatric hospital setting: a comprehensive genome analysis of isolates from Guayaquil, Ecuador. *BMC Genomics* 2024; 25: 928.

66. Sherry NL, Horan KA, Ballard SA, Gonçalves da Silva A, Gorrie CL, Schultz MB, *et al*. An ISO-certified genomics workflow for identification and surveillance of antimicrobial resistance. *Nat Commun* 2023; 14: 60.

FIGURES LEGENDS

Figure 1. Distribution of bacterial isolates by sample type and species. (left) Stacked bar chart showing the distribution of isolated

bacterial species, categorized by clinical sample type. The number of isolates per sample type is indicated in parentheses; (right) pie chart representing the overall proportion of bacterial species identified across all samples.

Figure 2. Antimicrobial Susceptibility Profiles. This bar chart depicts the antimicrobial susceptibility profiles of bacterial isolates (n = varies per antibiotic). The bars represent the percentage of isolates classified as susceptible (green), intermediate (yellow), and resistant (red) to each antibiotic tested. Antibiotics are ordered by the number of isolates tested. Results were interpreted according to CLSI 2023 guidelines.

Figure 3. Venn diagram showing the distribution of carbapenemase genes detected among the bacterial isolates. The analysed genes were *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP-1}$, and *bla*$_{OXA-48}$ (for simplicity, KPC, NDM, VIM, IMP-1, and OXA-48 in the figure). The overlapping regions indicate isolates harbouring multiple carbapenemase genes, highlighting combinations such as *bla*$_{KPC}$ and *bla*$_{NDM}$, *bla*$_{KPC}$ and *bla*$_{VIM}$, and *bla*$_{KPC}$ and *bla*$_{OXA-48}$. The absence of *bla*$_{IMP-1}$is reflected in its lack of representation in the diagram.

Figure 4. Gower distance and heatmap displaying phylogenetic relationships and antimicrobial susceptibility profiles of bacterial isolates. The isolates include *Klebsiella pneumoniae* and other species such as *Pseudomonas aeruginosa, Serratia marcescens, Acinetobacter baumannii,* and *Escherichia coli*. The heatmap colors indicate

antimicrobial susceptibility: red (resistant/positive genetic test), yellow (intermediate resistance), green (susceptible), and blue (negative genetic test). The scale at the bottom represents the phylogenetic distance between the isolates.

FIGURES

Figure 1.

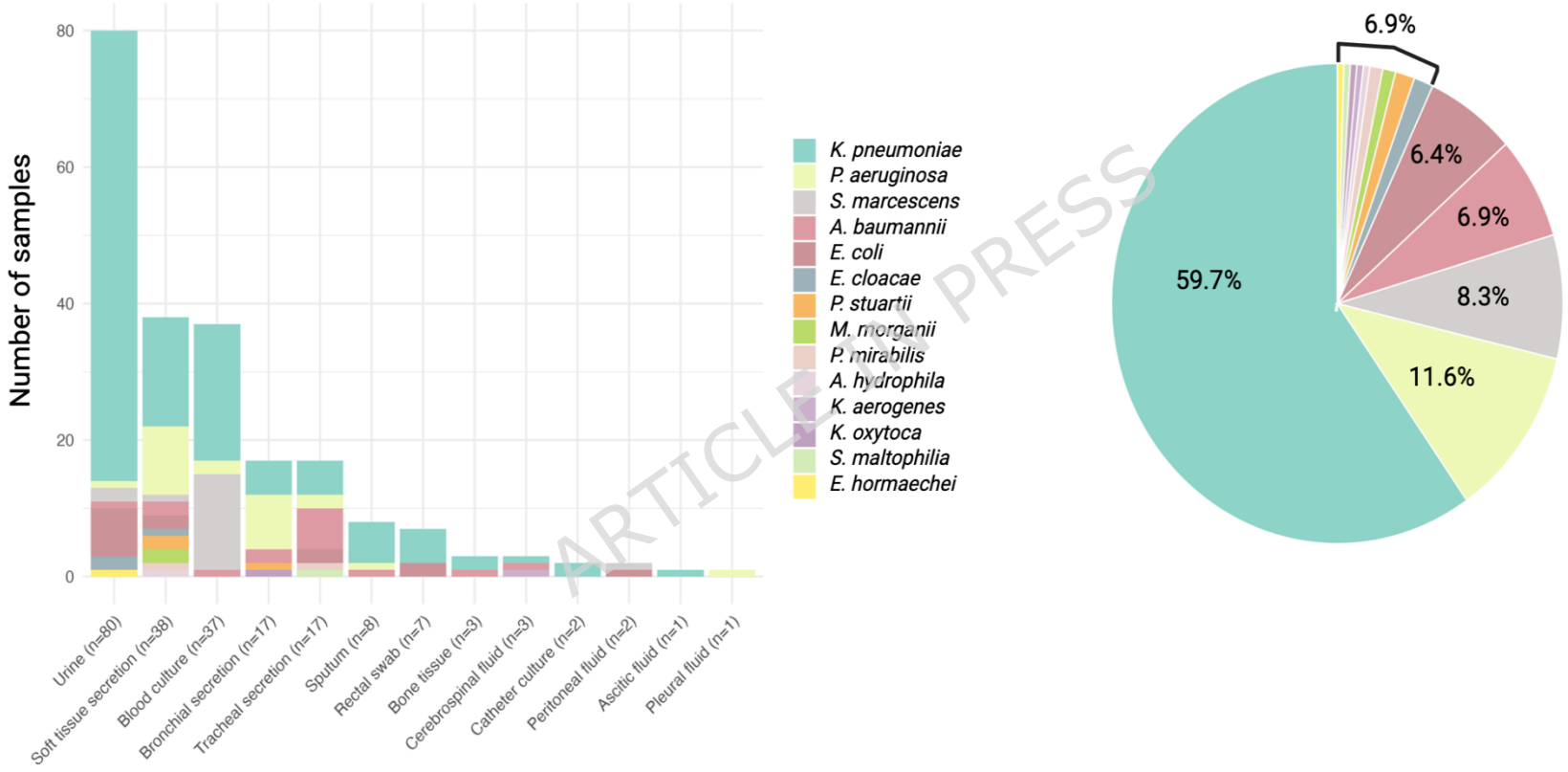


Figure 2.

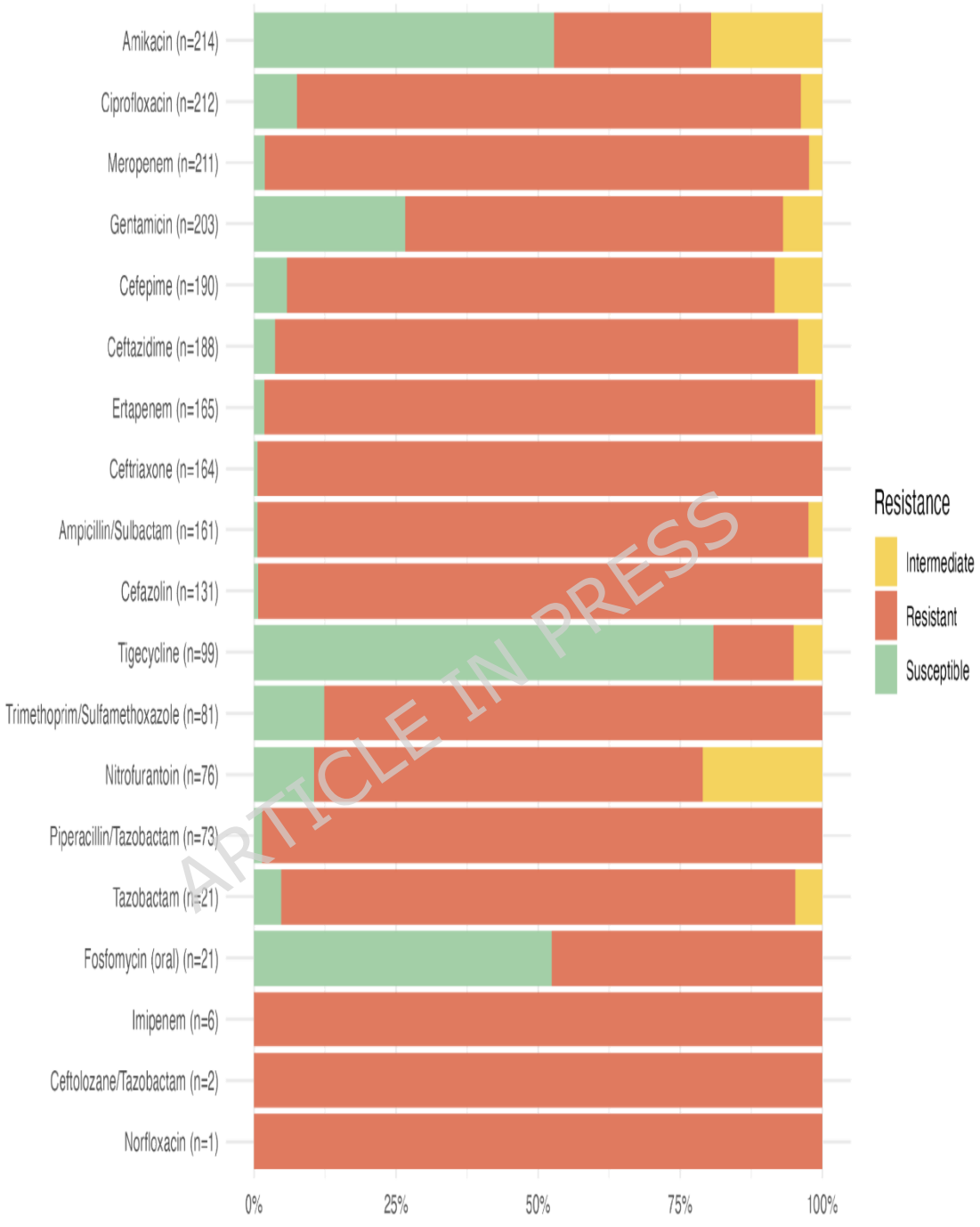


Figure 3.

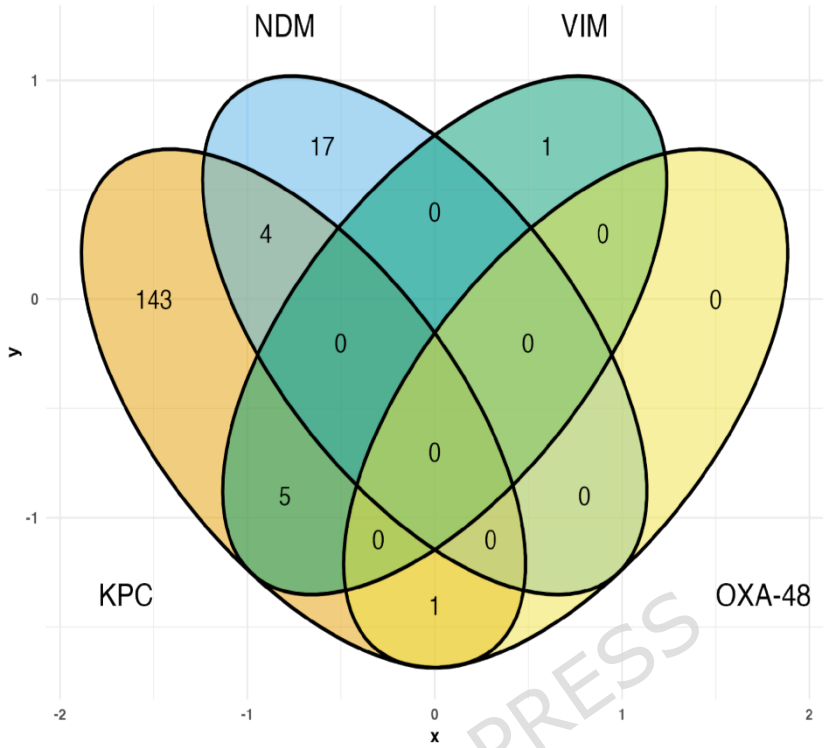
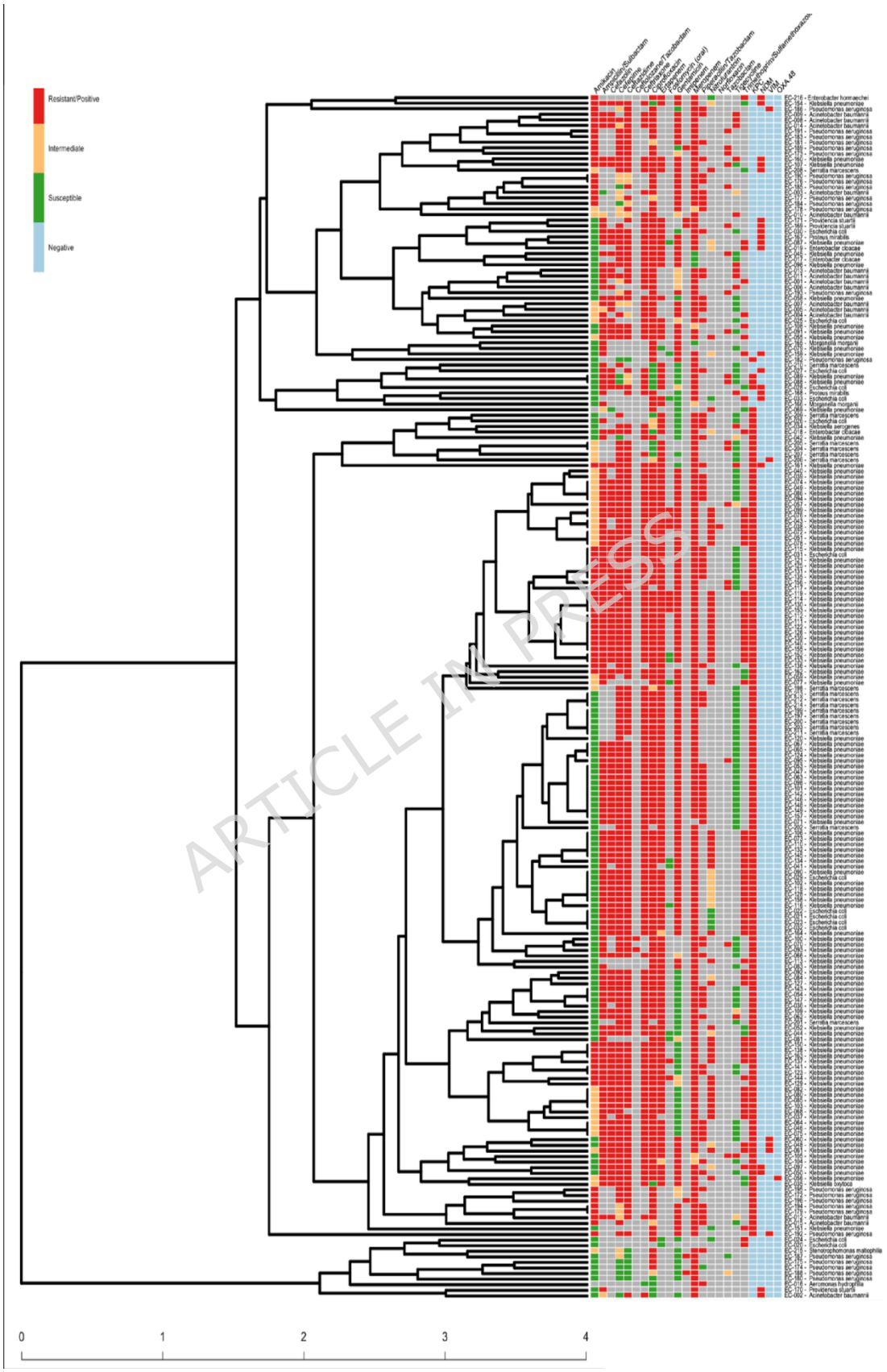


Figure 4.



Supplementary material

Table S1. TaqMan probes (Thermo Fisher Scientific, Waltham, MA, USA) were used to detect *bla*$_{KPC}$, *bla*$_{NDM}$, *bla*$_{VIM}$, *bla*$_{IMP-1}$, *bla*$_{OXA-48,}$ and *bla*$_{CTX-M}$ resistance genes.

Assay GEX1	**ASSAY ID**	**DYE**
bla$_{KPC}$	Ba04646152_S1	VIC - MGB
bla$_{NDM}$	Ba04931076_S1	FAM - MGB
bla$_{VIM}$	Ba04646155_S1	ABY - QSY
C-Human (control)	Hs01060665_g1	JUN - QSY

Assay GEX2	**ASSAY ID**	**DYE**
bla$_{IMP-1}$	Ba04646116_S1	FAM - MGB
bla$_{OXA-48}$	Ba04930816_S1	VIC - MGB
bla$_{CTX-M}$	Ba04646149_S1	ABY - QSY
C-Human (control)	Hs01060665_g1	JUN - QSY

Table S2. Isolates Carrying Carbapenemase Gene Combinations. (excel file)