



ORIGINAL ARTICLE

Antimicrobial Resistance of Enterobacteriaceae Isolated in Intensive Care Units of the Abel Santamaría Cuadrado Hospital

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ABSTRACT

Introduction: Intensive Care Units (ICUs) are hotspots for infections, where patients face heightened vulnerability due to routine invasive procedures and prolonged antimicrobial use, fostering antimicrobial resistance.

Objective: To characterize the antimicrobial resistance profiles of Enterobacteriaceae isolated in the ICUs of Abel Santamaría Cuadrado Hospital.

Methods: An observational, analytical, prospective cross-sectional study was conducted in the Microbiology Service of the "Abel Santamaría Cuadrado" General Teaching Hospital in Pinar del Río, Cuba, from January 2022 to July 2024. Absolute and relative frequencies were calculated.

Results: Of the 94 biological samples analyzed, 81,9 % were respiratory secretions, with only one sample corresponding to body fluids. Enterobacteriaceae were predominantly isolated from these. The most frequent resistance phenotype was plasmid-mediated AmpC β -lactamase production (23 cases), present across six genera. Mutations in the quinolone resistance-determining region (QRDR) predominated in *Proteus* and *Pantoea*, while extended-spectrum β -lactamase (ESBL) production was observed in *Providencia*. Antimicrobial resistance was universal: 100 % of isolates were resistant to at least one drug, 92,6 % were multidrug-resistant (MDR), and 14,9 % exhibited pandrug resistance (PDR)—resistance to all antimicrobial classes—highlighting a severe clinical problem.

Conclusions: Infections caused by Enterobacteriaceae carrying resistance phenotypes constitute a major source of severe morbidity among ICU patients.

Keywords: Critical Care; Enterobacteriaceae; Drug Resistance, Microbial; Intensive Care Units.

INTRODUCTION

In 2020, the World Health Organization (WHO) identified antimicrobial resistance (AMR) as a critical global health threat, particularly highlighting multidrug-resistant bacteria such as *Acinetobacter*, *Pseudomonas*, *Klebsiella*, and *Escherichia coli*. These pathogens have acquired resistance to numerous antibiotics, including carbapenems and third- and fourth-generation cephalosporins.⁽¹⁾

Enterobacteriaceae are a large, heterogeneous group of Gram-negative bacilli naturally residing in the intestinal tracts of humans and animals. The family includes genera such as *Escherichia*, *Shigella*, *Salmonella*, *Enterobacter*, *Klebsiella*, *Serratia*, and *Proteus*. While some, like *E. coli*, are part of the normal microbiota and cause opportunistic infections, others—such as *Salmonella* and *Shigella*—are primary human pathogens.^(2,3)

β -lactams are the most widely used antimicrobials globally for treating bacterial infections, including bacteremia. The primary mechanism of β -lactam resistance in Enterobacteriaceae is β -lactamase production. Carbapenems—considered last-resort agents against increasingly resistant Enterobacteriaceae—have been compromised by carbapenemases, which are often encoded on mobile genetic elements and spread horizontally. Over the past decade, outbreaks of carbapenemase-producing Enterobacteriaceae (CPE) have been reported worldwide, including in Latin America.^(2,4)

Multidrug resistance in Enterobacteriaceae is strongly associated with acquired β -lactamases—particularly extended-spectrum β -lactamases (ESBLs), plasmid-mediated AmpC (class C) enzymes, and carbapenemases.⁽⁵⁾ Risk factors for CPE colonization or infection include advanced age, disease severity, ICU admission, prior antimicrobial exposure (especially carbapenems, quinolones, and cephalosporins), and invasive procedures (e.g., endoscopy, prolonged central venous catheterization).⁽⁶⁾

ICUs are epicenters of infection in clinical practice. Critically ill patients are exceptionally vulnerable due to routine invasive interventions and prolonged drug exposure, creating ideal conditions for AMR emergence. Managing these infections is complex, given the high morbidity and mortality and the escalating challenge of antimicrobial resistance.⁽⁷⁾

Standardized definitions are essential for AMR surveillance: Multidrug-resistant (MDR): nonsusceptibility to ≥ 1 agent in ≥ 3 antimicrobial categories. Extensively drug-resistant (XDR): nonsusceptibility to all but one or two categories. Pandrug-resistant (PDR): resistance to all agents in all categories. These definitions facilitate global comparability of epidemiological data and inform public health strategies.⁽⁸⁾

ICU-acquired infections are further driven by factors such as advanced age, comorbidities, immunosuppression, prolonged hospitalization, and antimicrobial selective pressure.^(9,10)

Urgent actions—including strict antimicrobial stewardship, development of novel therapeutics, and robust infection surveillance—are critical to curb resistance. This study aimed to characterize the antimicrobial resistance profiles of Enterobacteriaceae isolated from ICU patients at Abel Santamaría Cuadrado Hospital to inform local clinical and policy responses.

METHODS

An observational, analytical, prospective cross-sectional study was conducted in the Microbiology Service of the "Abel Santamaría Cuadrado" General Teaching Hospital in Pinar del Río, Cuba, from January 2022 to July 2024.

The universe was represented by all the microbiological samples analyzed in the miscellaneous department corresponding to patients admitted to the intensive care units of the General Teaching Hospital "Abel Santamaría Cuadrado" during the study period in the years 2023 and 2024.

The sample was represented by all the microbiological specimens from the universe that tested positive for enterobacteria. For this, compliance with the inclusion criteria was taken into account (microbiological samples that tested positive for enterobacteria and for which all the required microbiological studies in the research could be performed).

Data collection methods: Empirical (observation, documentary review), theoretical (analysis and synthesis), and historical-logical approaches were employed. Primary data were extracted from the Microbiology Service's database.

The biological samples were collected by a microbiology technician or graduate, employing all recommended aseptic and antiseptic procedures, as well as the sample collection method indicated for the isolation sites under study.

Samples processed included respiratory secretions from the endotracheal tube of ventilated patients, pleural drain cultures from patients with pleurotomy, cultures of central venous catheter tips, skin lesions, surgical wounds, body fluids (pleural, peritoneal), and purulent secretions

The study adhered to the four core ethical principles-respect for persons, beneficence, non-maleficence, and justice-and complied with the Declaration of Helsinki (1975, revised 1983 and 2000). Hospital administration granted formal authorization, and the study was approved by the Scientific Council and Ethics Committee of the "Abel Santamaría Cuadrado" General Teaching Hospital.

RESULTS

The type of biological sample analyzed in relation to the bacterial genus is shown in Table 1. Of the 94 biological samples studied, 77 (81,9 %) were respiratory secretions, making this the predominant sample type in the study and the source in which each Enterobacteriaceae species included in the investigation was most frequently isolated. Only one biological sample consisted of body fluids.

Table 1. Distribution of cases according to biological sample type and Enterobacteriaceae genus observed.

Sample type	Genre													
	Esch		Entb		Kleb		Prot		Citr		Pant		Prov	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
Respiratory secretion	3	3,2	36	38,3	3	3,2	6	6,4	13	13,8	15	16,0	1	1,1
Catheter tip	1	1,1	3	3,2	0	0	0	0	2	2,1	2	2,1	0	0
Purulent secretion	1	1,1	3	3,2	0	0	0	0	1	1,1	0	0	0	0
Body Fluids	0	0	0	0	0	0	0	0	0	0	1	1,1	0	0
Surgical wounds	1	1,1	0	0	0	0	2	2,1	0	0	0	0	0	0

Legend: Esch: *Escherichia*; Entb: *Enterobacter*; Kleb: *Klebsiella*; Prot: *Proteus*; Citr: *Citrobacter*; Pant: *Pantoea*; Prov: *Providencia*

In **Table 2**, it can be observed that the phenotype characterized by the production of plasmid-mediated AmpC β -lactamase was the most frequent in the study (23 biological samples) and was present in 6 of the 7 Enterobacteriaceae genera included in the investigation. Specifically, in *Proteus* and *Pantoea*, the phenotype associated with mutations in the quinolone resistance-determining region (QRDR) was more commonly observed. In the genus *Providencia*, only the phenotype characterized by the production of extended-spectrum β -lactamases (ESBLs) was detected.

Table 2. Resistance phenotypes by bacterial genus observed.

Resistance phenotypes	Genre													
	Esch		Entb		Kleb		Prot		Citr		Pant		Prov	
	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%	No.	%
AmpC	2	2,1	10	10,6	2	2,1	3	3,2	5	5,3	1	1,1	0	0
BLEA	1	1,1	3	3,2	0	0	0	0	1	1,1	3	3,2	1	1,1
BLEE	1	1,1	4	4,3	0	0	1	1,1	3	3,2	5	5,3	0	0
MBL	0	0	3	3,2	1	1,1	0	0	0	0	2	2,1	0	0
KPC	0	0	1	1,1	0	0	0	0	0	0	0	0	0	0
QRDR	2	2,1	5	5,3	0	0	4	4,3	5	5,3	6	6,4	0	0

Legend: Esch: *Escherichia*; Entb: *Enterobacter*; Kleb: *Klebsiella*; Prot: *Proteus*; Citr: *Citrobacter*; Pant: *Pantoea*; Prov: *Providencia*; AmpC: plasmid-mediated AmpC β -lactamase; BLEA: broad-spectrum β -lactamase; BLEE: extended-spectrum β -lactamase (ESBL); MBL: metallo- β -lactamase; KPC: *Klebsiella pneumoniae* carbapenemase; QRDR: quinolone resistance-determining region.

Antimicrobial resistance among Enterobacteriaceae is presented according to established antimicrobial resistance patterns. The results, summarized in **Table 3**, show that drug resistance was present in 100 % of cases, as all isolates demonstrated resistance to at least one antimicrobial agent. Multidrug resistance (MDR)—defined as resistance to at least one antimicrobial from three or more pharmacological classes—was observed in 92,6 % of cases. Furthermore, 14,9 % of isolates exhibited pandrug resistance (PDR), meaning they were resistant to all antimicrobials across all categories.

Table 3. Distribution of the sample according to antimicrobial resistance patterns.

Resistance patterns	No.	%
Drug resistance (DR)	94	100
Multidrug resistance (MDR)	87	92,6
Extensively drug-resistant (XDR)	41	43,6
Pandrug-resistant (PDR)	14	14,9

DISCUSSION

Antimicrobial resistance is defined as the ability of a microorganism to withstand the effects of antibiotics; it may be an inherent characteristic of the bacterium or an acquired trait developed during the infectious process.⁽¹¹⁾ The current level of microbial resistance to antimicrobials represents a serious global health problem and a major challenge for the future. Research has elucidated the mechanisms and causes underlying this resistance, as well as strategies involving both new and existing pharmaceutical agents to counteract it.^(12,13)

Hoo et al.⁽¹⁴⁾ reported a predominance of Gram-negative bacteria, with *Klebsiella* spp. being the most frequently isolated (63,8 %), particularly in respiratory secretions—consistent with our findings, where *Klebsiella*-positive samples were also obtained from respiratory secretions. Other authors have similarly identified *E. coli* and *K. pneumoniae* as the most common isolates in respiratory specimens.⁽¹⁵⁾ Publications on the topic confirm that these bacteria are frequently recovered from the respiratory tract.⁽¹⁶⁾

Reviewed studies indicate that central line-associated bloodstream infections are predominantly caused by Gram-positive cocci.^(17,18) This may explain our observation that few catheter-tip samples yielded Enterobacteriaceae. Other sample types were underrepresented in our study.

Gram-negative bacteria are notorious for their extensive diversity and adaptive modifications, which have enabled resistance to many currently available antibiotics. Four primary mechanisms underlie bacterial antimicrobial resistance:

- **Hydrolytic enzymes:** Some bacteria produce enzymes (e.g., β -lactamases) that degrade antibiotics, neutralizing their effect.
- **Target site mutation:** Bacteria alter specific amino acids at the antibiotic's binding site, eliminating its molecular target.
- **Membrane impermeability:** Modifications in porin size or number in the outer membrane restrict antibiotic entry into the cytosol.
- **Antibiotic efflux:** Bacteria employ efflux pumps to actively expel antibiotics from the cell, treating them as waste or toxins.⁽¹⁹⁾

The presence of multidrug-resistant pathogens is widely recognized as a major global public health threat with significant socioeconomic impact. Approximately 700,000 deaths worldwide are attributed annually to antimicrobial resistance; without immediate and effective implementation of WHO-recommended measures, this figure could rise to 10 million deaths per year by 2050.⁽²⁰⁾

Although most resistance genes exist naturally, antibiotic administration inevitably selects for resistant strains. While antimicrobial resistance is a natural evolutionary process, the misuse and overuse of antibiotics in human medicine have drastically accelerated its development and spread—evident in our study. β -lactams are bactericidal antibiotics that inhibit the final stage of peptidoglycan synthesis by competitively binding to penicillin-binding proteins (PBPs). In some bacterial species, the expression of multiple PBPs contributes to resistance against most β -lactams, complicating treatment—clearly reflected in our results. ⁽²¹⁾

Certain Enterobacteriaceae naturally produce chromosomally encoded AmpC β -lactamases—including *Enterobacter* spp., *Providencia* spp., *Morganella morganii*, *Serratia marcescens*, *Citrobacter freundii*, and *Hafnia alvei*. In contrast, *Escherichia coli* possesses non-inducible chromosomal AmpC genes, but their basal expression is typically low. ⁽²²⁾

Extended-spectrum β -lactamase (ESBL)-producing bacteria have emerged as a major concern in both hospitalized and community settings. Over the past five years, global ESBL-producing Enterobacteriaceae isolation rates have surged, reaching up to 55 % in China and 79 % in India. Metallo- β -lactamases (MBLs) represent the most diverse class of carbapenemases, with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* being the primary carriers. ⁽²¹⁾

Notably, half of the *E. coli* and *K. pneumoniae* isolates in one study exhibited ESBL production, with meropenem resistance observed in 20 % of cases. ⁽¹⁵⁾ ESBL and CRE colonization showed high prevalence in emergency department patient cultures. ⁽²³⁾ In our study, KPC (*Klebsiella pneumoniae* carbapenemase) was detected in only one isolate. In contrast, Lipari et al. ⁽²⁾ reported a high predominance of KPC-producing *K. pneumoniae*, associated with an attributable mortality of 52,4 %.

These high rates of drug resistance and multidrug resistance are largely driven by phenotypic adaptations in bacteria—particularly due to frequent, indiscriminate antibiotic exposure. The MDR profiles exhibited by ESBL-producing strains pose significant therapeutic challenges in both hospital and community settings. ⁽²¹⁾ A study in Mexico found that 78 % of Gram-negative and 69% of Gram-positive isolates displayed MDR, XDR, or PDR patterns, with *E. coli* and *Klebsiella* spp. among the most common MDR Gram-negative pathogens. ⁽⁸⁾ Other researchers identified *E. coli* as a leading cause of MDR in New Zealand, primarily in urinary tract infections. ⁽²⁴⁾ Espinosa et al. ⁽²⁵⁾ reported that 57 % of Enterobacteriaceae strains were multidrug-resistant, linking this trend to inappropriate antibiotic use as a key driver of resistance mechanisms.

To design and implement measurable, targeted interventions, it is essential to transform antimicrobial resistance data into specific, actionable knowledge regarding the characteristics and magnitude of the problem—as demonstrated in this study.

CONCLUSIONS

The alarming resistance rates identified underscore the urgent need for more rational antibiotic use in intensive care units. Continuous surveillance of infectious etiologies and antimicrobial resistance patterns in hospitals is essential to guide future therapeutic decisions.

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