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Trends in Antimicrobial Resistance Mechanisms and Susceptibility among Enterobacterales in Hospitalized Patients: A Single Center Microbiological Clinical Analysis 2010-2025

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Abstract

Background: To evaluate trends in antimicrobial resistance mechanisms among *Enterobacterales* over a sixteen-year period. **Methods:** Data for *Enterobacterales* isolates were extracted from a VITEK® 2 Compact system database. Trends, resistance mechanisms, and susceptibilities were analyzed for extended-spectrum β-lactamase (ESBL), carbapenem-resistant *Enterobacterales* (CRE), and AmpC-producing strains. Phenotypic resistance patterns were checked manually for selected strains to ensure interpretation consistency. **Results:** A total of 20,266 *Enterobacterales* isolates were analyzed, comprising *Escherichia coli* (n = 12,783; 63.1%), *Klebsiella* (n = 4,302; 21.2%), *Enterobacter* (n = 1,060; 5.23%), Proteeae (n = 1,426; 7.04%), *Citrobacter* (n = 391; 1.93%), and *Serratia* (n = 304; 1.5%). The overall prevalence of ESBL-producing *Enterobacterales* (ESBL-PE) was 29.8%, *E. coli* 47.0%, *Klebsiella* 36.0%, and 5.3% for Proteeae. AmpC production was observed in 13.9% as an overall, for *Enterobacter* 25.0%. *Klebsiella* 18.0%, *Serratia* 19%, *E. coli* 13%, *Citrobacter* 9.0%, and 6.0% of Proteeae. SHV-1 hyperproduction was identified in 8.85%, *E. coli* 13.0%, and 1.9% of *Klebsiella*. The overall prevalence of CRE was 22.4%, of which carbapenemase production was detected in 25.0% of *Klebsiella*, *Enterobacter* 11%, *Serratia* 5.3%, *Citrobacter* 4.9%, *E. coli* 3.0%, and 2.3% of Proteeae. Porin impermeability was found in 20.3% of *Klebsiella*, *Enterobacter* 10.1%, and 2.5% of *E. coli*. Metallo-β-lactamases (MβLs) or *K. pneu-*

moniae carbapenemase (KPC) expression was present in 2.4% of *Klebsiella*, and <1.0% of *Enterobacter*, *E. coli* and *Citrobacter*. TEM-or-OXA mediated resistance was noted in *Klebsiella* 17.0%, *E. coli* 16%, and 11.0% of Proteeae. **Conclusion:** Trends for ESBL-PE, CRE, AmpC-producers, and SHV-1 hyperproducers showed minimal chronological changes over the 16-year period, which were not clinically significant (OR = 1.0, P < 0.001). Susceptibility among ESBL-PE isolates to relatively newer antimicrobial agents ranged was not available, while CRE susceptibility ranged from 31.0% to 32.0%.

Keywords

ESBL-PE, CRE, AmpC-Producers, SHV1, Carbapenemases

1. Introduction

The global escalation of antimicrobial resistance (AMR) among Gram-negative bacilli appears relentless. Across various countries, strategies to halt, mitigate, or reverse this trajectory are being evaluated through diverse frameworks, particularly within geographical hotspots such as low- and middle-income countries (LMICs) [1] [2]. Members of the order *Enterobacterales* represent the most frequent pathogens associated with healthcare-associated infections (HAIs) and constitute a critical global public health threat. Furthermore, they account for a substantial and increasing proportion of community-associated infections, characterized by a steady trajectory of escalating resistance.

Public health attention shifted toward community-acquired resistant *Enterobacterales* in the 1990s as resistance rates to essential antibacterials accelerated at an alarming pace. This trend ultimately prompted the World Health Organization (WHO) to prioritize the discovery and development of novel antimicrobials to address these expanding clinical challenges [3]-[5].

Within healthcare facilities, environmental surfaces and patient fomites frequently become colonized with *Enterobacterales* and other bacterial classes, presenting an immediate transmission risk to patients, an essential prerequisite for the development of clinical nosocomial infections. Severely predisposed patient populations face the highest risk for *Enterobacterales* infection, including individuals with hematopoietic malignancies, those undergoing cytotoxic chemotherapy, patients infected with atypical pathogens (e.g., nontuberculous mycobacteria), solid-organ transplant recipients, patients undergoing invasive or interventional procedures, and individuals with multiple severe comorbidities. Concurrently, patients without defined risk factors who are admitted for alternative indications also remain vulnerable to colonization and infection [6]-[8].

Escherichia coli, *Klebsiella pneumoniae*, and *Enterobacter* species serve as the primary etiologic agents within this order, frequently causing severe pulmonary, intra-abdominal, and urinary tract infections, and less commonly, cutaneous or

central nervous system infections. In contrast, while members of the tribe Proteeae can cause infections like other *Enterobacterales* though uncommonly cause blood stream infection.

Infections driven by these pathogens can rapidly progress to severe sepsis, septic shock, and multiple organ dysfunction syndrome (MODS), culminating in high mortality rates. Critically, multidrug-resistant phenotypes, such as carbapenem-resistant *Enterobacterales* (CRE), demonstrate even higher attributable mortality rates among immunocompromised and critically ill populations [9] [10].

The primary objective of this longitudinal study is to characterize the localized burden, resistance patterns, rates, and chronological trends of *Enterobacterales* over a 16-year period within this region. In doing so, we aim to delineate the therapeutic implications for clinical practice, evaluate the efficacy of recently introduced combination antimicrobial agents against resistant strains—ceftazidime-avibactam and ceftolozane-tazobactam were introduced in the year 2021, and meropenem-vaborbactam was introduced in 2024 and underscore the urgent need for action within LMICs [11]. In these settings, effective antimicrobial stewardship programs (ASPs) frequently remain under-resourced, selectively implemented, or altogether absent.

2. Materials and Methods

2.1. Microbiological Identification and Antimicrobial Susceptibility Testing

Clinical specimens were processed according to standard microbiological procedures. Specimens were inoculated onto routine culture media, including blood agar and MacConkey agar, and incubated at 35˚C - 37˚C for 18 h - 24 h. Lactose-fermenting Gram-negative bacilli consistent with the order *Enterobacterales* were selected based on colony morphology, Gram stain, and oxidase negativity.

2.2. Identification of Enterobacterales

Bacterial identification was performed using VITEK® 2 compact system (bioMérieux, Marcy-L'Etoile, France). A pure colony from a fresh culture was suspended in sterile 0.45% - 0.50% saline, and turbidity was adjusted to a 0.50 McFarland standard using a calibrated densitometer. The Gram-Negative identification card was inoculated according to the manufacturer's instructions and loaded into the instrument. The VITEK® 2 system incubated and interpreted the card automatically, generating species-level identification based on biochemical reaction profiles. Identification results were accepted when the VITEK® 2 system indicated an acceptable "confidence level".

2.3. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing (AST) was conducted using VITEK® 2 AST

cards appropriate for Gram-negative organisms. A standardized suspension (0.50 McFarland) from a pure isolate was prepared in sterile saline and used to inoculate AST cards following the manufacturer's instructions. The cards were incubated and read automatically by the instrument, which generated minimum inhibitory concentration (MIC) values or categorical interpretations depending on the antimicrobial agent. Susceptibility results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) performance standards current for the year of testing. CLSI categorical breakpoints (susceptible, intermediate, and resistant) were used for all antimicrobial agents studied. Intrinsic resistances and non-reportable agent-organism combinations were identified and excluded from analysis in accordance with CLSI guidelines.

2.4. Quality Control

Quality control (QC) procedures were performed following CLSI recommendations using standard reference strains, including *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853. QC was conducted with each new lot of cards and at the frequency of once weekly. AST results were accepted only when QC values fell within CLSI-specified acceptable ranges.

2.5. VITEK® 2 as a Method to Detect Resistant Mechanisms

This study delineates the phenotypic characteristics and antimicrobial susceptibility profiles of resistant *Enterobacterales* strains, specifically extended-spectrum *β*-lactamase-producing *Enterobacterales* (ESBL-PE) and their associated phenotypes, AmpC-producers, and carbapenem-resistant *Enterobacterales* (CRE), utilizing the automated VITEK® 2 system (bioMérieux, Marcy-L'Etoile, France). The VITEK® 2 platform has previously been validated as a reliable diagnostic tool for identifying resistant strains with excellent accuracy. For instance, a South African study evaluated the sensitivity and specificity of the VITEK® 2 system for ESBL-PE detection against standard disk diffusion and broth microdilution methods, coupled with conventional polymerase chain reaction (PCR) assays for $bla_{CTX\text{-}M}$, bla_{SHV}, and bla_{TEM} genes. In that evaluation, the VITEK® 2 system demonstrated diagnostic sensitivities for ESBL-PE detection of 92% for Escherichia coli and 100% for Klebsiella pneumoniae, with corresponding specificities of 100% and 90%, respectively. However, that study noted potential diagnostic misinterpretations when evaluating SHV-hyperproducing K. pneumoniae isolates [12].

Concurrently, a multi-center evaluation in North and Latin America compared the VITEK® 2 Advanced Expert System (AES) against genotypic results obtained via whole-genome sequencing (WGS) across 488 resistant *Enterobacterales* phenotypes. The VITEK® 2 AES provided phenotypic profiles for 447 (91.6%) isolates, capturing carbapenemases, ESBLs, and plasmid-mediated AmpC (tAmpC) genes, alongside wild-type strains. Overall, the AES phenotypic predictions were correct for 96.9% of the isolates. Specifically, the system accurately identified car-

bapenemase phenotypes with an overall accuracy of 93.7% (sensitivity: 96.4%; specificity: 91.7%). ESBL-PE phenotypes with an accuracy of 93.7% (sensitivity: 98.1%; specificity: 92.4%). tAmpC phenotypes with an accuracy of 98.4% (sensitivity: 82.1%; specificity: 99.5%). and wild-type isolates with a sensitivity of 100% and specificity of 98.8%. Consequently, these automated phenotypic profiles provide highly reliable therapeutic guidance and significantly reinforce clinical antimicrobial stewardship programs (ASPs) [13].

2.6. Data Handling and Selection Criteria

Only non-duplicate clinical isolates obtained for routine diagnostic purposes were eligible for inclusion. To eliminate bias from repetitive sampling, bacteria included in the final analysis were only the index (first) isolate per a patient, a colonizer, or a pathogen for the first distinct infection episode. Antimicrobial susceptibility testing (AST) data were extracted from the laboratory information system (LIS) and categorized according to the Clinical and Laboratory Standards Institute (CLSI) interpretive breakpoints corresponding to each respective study year. Intermediate isolates were grouped together with resistant strains for analysis. Isolates categorized as susceptible-dose dependent (SDD) were grouped with susceptible strains; however, the total counts for both categories were negligible.

2.7. Data Collection and Variables

The laboratory receives and process specimens for the inpatients, spanning the different anatomical sources. All specimens are processed for identification and antimicrobial susceptibility testing by VITEK® 2 automated system (*bioMérieux, Marcy-L'Etoile, France*). The system maintains a multi-year database with a backup repository. Data was exported as text files and formatted into Microsoft Excel sheet (*Microsoft Corporation*). Patients related metadata collected included hospital location, specimen type, specimen source, specimen date, collection date, testing date, organism name, its bio-number, probability and confidence level. Bacteria isolate-related variables included AST, minimum inhibitory concentrations (MICs), predicted resistance enzyme profiles, and additional Advanced Expert System (AES) algorithmic interpretations of resistance mechanisms across distinct antimicrobials classes.

2.8. Ethics Statement

The Institutional Review Board (IRB) of The Specialty Hospital reviewed and formally approved the study protocol (Approval No. IRB 125771/T/5 issued on 8 June 2026). Because this study did not involve direct human subject participation and relied exclusively on de-identified, historical microbiological data retrieved from the archived VITEK® 2 database, the requirement for informed consent was waived.

2.9. Statistical Analysis

The master data frame was converted to a comma-separated values (CSV) format

and imported into R version 4.5.2 (*R Core Team* (*2025*). *R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL* https://www.R-project.org/.) via the RStudio Integrated Development Environment (*Posit team* (*2025*). *RStudio: Integrated Development Environment for R. Posit Software, PBC, Boston, MA. URL* http://www.posit.co/.). Data cleaning, manipulation, and structural adjustments were executed primarily within the "tidyverse" framework. A target subset containing the *Enterobacterales* isolates of interest was generated for primary analysis. Descriptive statistics, including frequency distributions, cross-tabulations, and graphical visualizations, were used to characterize baseline resistance patterns, annual isolate distribution rates, and temporal dynamics. To model trends in antimicrobial resistance over the 16-year study period, a generalized linear model (GLM) with a binomial logit link distribution was applied. Logistic regression coefficients were exponentiated to calculate odds ratios (ORs) and their corresponding 95% confidence intervals (CIs). Two-tailed p-values were calculated, and a level of < 0.05 was established as the threshold for statistical significance.

3. Results

3.1. Isolate Distribution and Baseline Characteristics

Table 1. Detected resistance mechanisms among common clinical isolates of *Enterobacterales*.

Resistance Phenotype*	*E. coli* N = 12,783[1]	Klebsiella N = 4,302[1]	Enterobacter N = 1,060[1]	Proteeae N = 1,426[1]	Serratia N = 304[1]	Citrobacter N = 391[1]
Identified types of the Extended Spectrum β-lactamases producers (ESβL)						
ESβL (all strains)	4,920 (47%)	1,124 (36%)	0 (NA%)	0 (NA%)	0 (NA%)	0 (NA%)
ESβL CTX-M LIKE	597 (4.7%)	122 (2.8%)	18 (1.7%)	76 (5.3%)	12 (3.9%)	11 (2.8%)
AmpC-producers	1,604 (13%)	754 (18%)	269 (25%)	86 (6.0%)	59 (19%)	35 (9.0%)
SHV1 hyperproduction	1,712 (13%)	81 (1.9%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Carbapenem-Resistant *Enterobacterales* Phenotypes						
Carbapenems (all strains)	377 (3.0%)	1,058 (25%)	122 (12%)	31 (2.3%)	18 (6.4%)	18 (5.2%)
Carbapenemases	383 (3.0%)	1,068 (25%)	115 (11%)	33 (2.3%)	16 (5.3%)	19 (4.9%)
Impermeability	321 (2.5%)	871 (20%)	107 (10%)	0 (0%)	0 (0%)	0 (0%)
MβL or KPC	18 (0.1%)	105 (2.4%)	10 (0.9%)	0 (0%)	0 (0%)	1 (0.3%)
TEM or OXA	1,994 (16%)	718 (17%)	0 (0%)	163 (11%)	0 (0%)	0 (0%)

Note: [1,*]**Resistance Phenotype:** note that the calculations in the table excluded columns counts with NA values as in *E. coli*. **1:** Total count per tested species. **ESBL:** Extended Spectrum, B-Lactamases. **CRE**: Carbapenem-Resistant *Enterobacterales*. **IRT or OXA:** Inhibitor-Resistant TEM (IRT) or OXA-type enzymes. PCR or Whole Genome Sequencing was not done to detect the specific resistance gene (blaTEM, blaOXA-1, blaOXA-48, etc.). Molecular method to detect the specific resistance genes (blaKPC, blaNDM, blaIMP, blaVIM, etc.) is needed for a more specific sub type. **Impermeabiltiy:** impermeable to cabrbapenems. **MβL or KPC:** *Klebsiella pneumoniae* metalo-B-lactamases. **TEM or OXA:** Inhibitor resistance Temonarian or Klebsiella pneumonia carbapenemases. **SHV1_Hyperproduction:** Sulphhydril variable causes resistance to penicillins and cephalosporine and affects Ceftazidime (ESBL-like), NA; not tested.

A total of 34,974 bacterial isolates were collected during the 16-year study period. Of these, Gram-negative bacilli accounted for 25,268 isolates, with members of the order *Enterobacterales* comprising 20,266 (80.2%) of the Gram-negative cohort.

Among the *Enterobacterales* {**denominator**} isolates, *Escherichia coli* was the most prevalent species (n = 12,783; 63.1%), followed by *Klebsiella* species—predominantly *Klebsiella pneumoniae* (n = 4,302; 21.2%), Proteeae species (n = 1,426; 7.0%), *Enterobacter* species (n = 1,060; 5.2%), *Citrobacter* species (n = 391; 2.8%), and *Serratia* species (n = 304; 3.9%). Phenotypic resistance profiles were evaluated both as standalone mechanisms and as co-existing resistance combinations (Table 1).

3.2. Extended-Spectrum β-Lactamase-Producing Enterobacterales (ESBL-PE)

The overall prevalence of ESBL-PE was 29.8%, primarily driven by *Escherichia coli* at 47.0% (including 4.7% $bla_{CTX\text{-}M\text{-}like}$ variants) and *Klebsiella* species at 36% (including 2.8% $bla_{CTX\text{-}M\text{-}like}$ variants), with *Klebsiella pneumoniae* as the dominant species. Minor proportions were identified among *Enterobacter* species 1.7%, Proteeae 5.3%, *Citrobacter* species 2.8%, and *Serratia* species 3.9%, all of which expressed $bla_{CTX\text{-}M\text{-}like}$ ESBL phenotypes (Table 1). Longitudinal analysis revealed a stable, minimal downward trend in ESBL-PE prevalence (Figure 1); however, while mathematically significant, the clinical effect size was negligible for both primary organisms (OR = 0.968, SE < 0.006, P < 0.001).

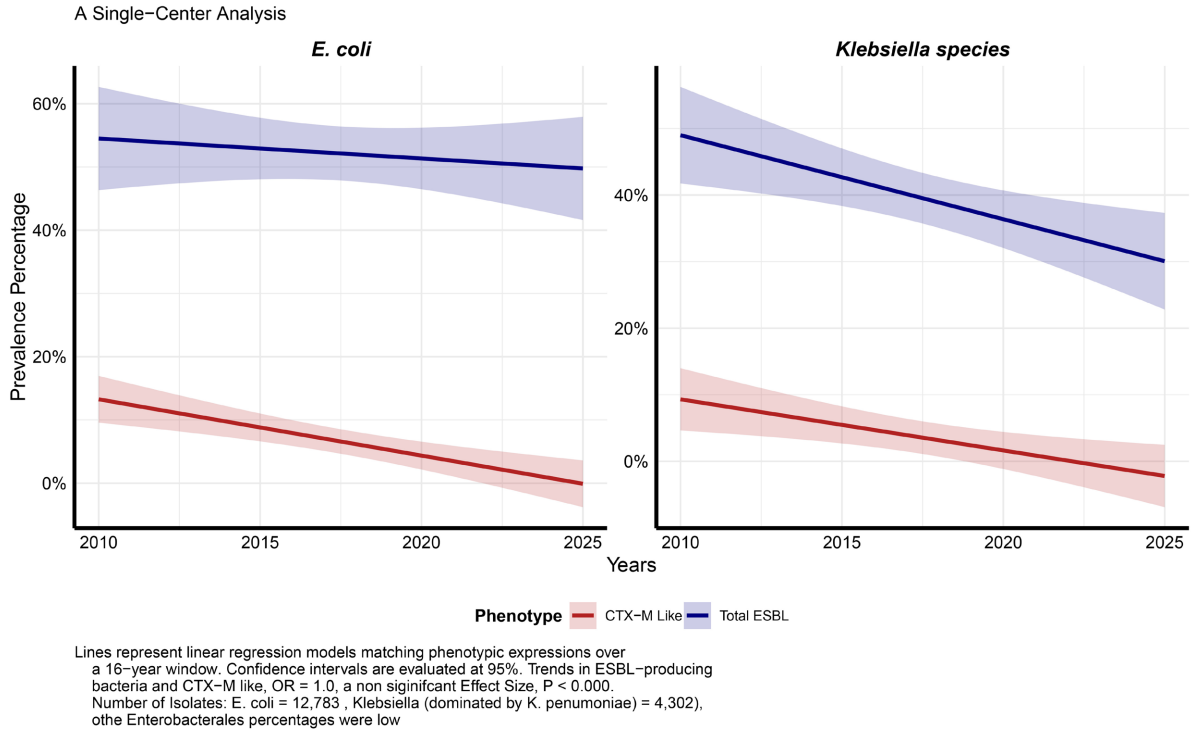

Figure 1. Trends in ESBL and CTX-M expression across *Enterobacterales* (2010-2025).

3.3. AmpC β-Lactamase Production

Phenotypic AmpC β-lactamase production was 13.9% of the total *Enterobacterales* cohort. Across individual genera, AmpC production rates were highest in *Enterobacter* species 25.0%, followed by *Klebsiella* species 18.0%, *Serratia* species 19.0%, *E. coli* 13.0%, *Citrobacter* species 9.0%, and Proteeae species 6.0% (Table 1).

Chronological trend analysis revealed distinct, divergent paths among specific genera: *Klebsiella* species demonstrated no trend over time (OR = 1.0, SE = 0.010, P < 0.000), whereas *Serratia* species displayed a progressive decline (OR = 0.881, SE = 0.037, P = 0.000; Figure 2(a)). *E. coli* showed no trends (OR = 1.0, SE = 0.00, P < 0.000). *Enterobacter* (OR = 0.982, SE = 0.017, P = 0.29). *Citrobacter* (OR = 0.912, SE = 0.048, P = 0.056). Proteeae (OR = 0.906, SE = 0.031, P = 0.002). Cumulatively, the overall trajectory for AmpC-producing strains minimally trending up throughout the study period, showing a significant though a minor and clinically negligible effect size (R = 1.04, SE = 0.006, P < 0.000; Figure 2(b)).

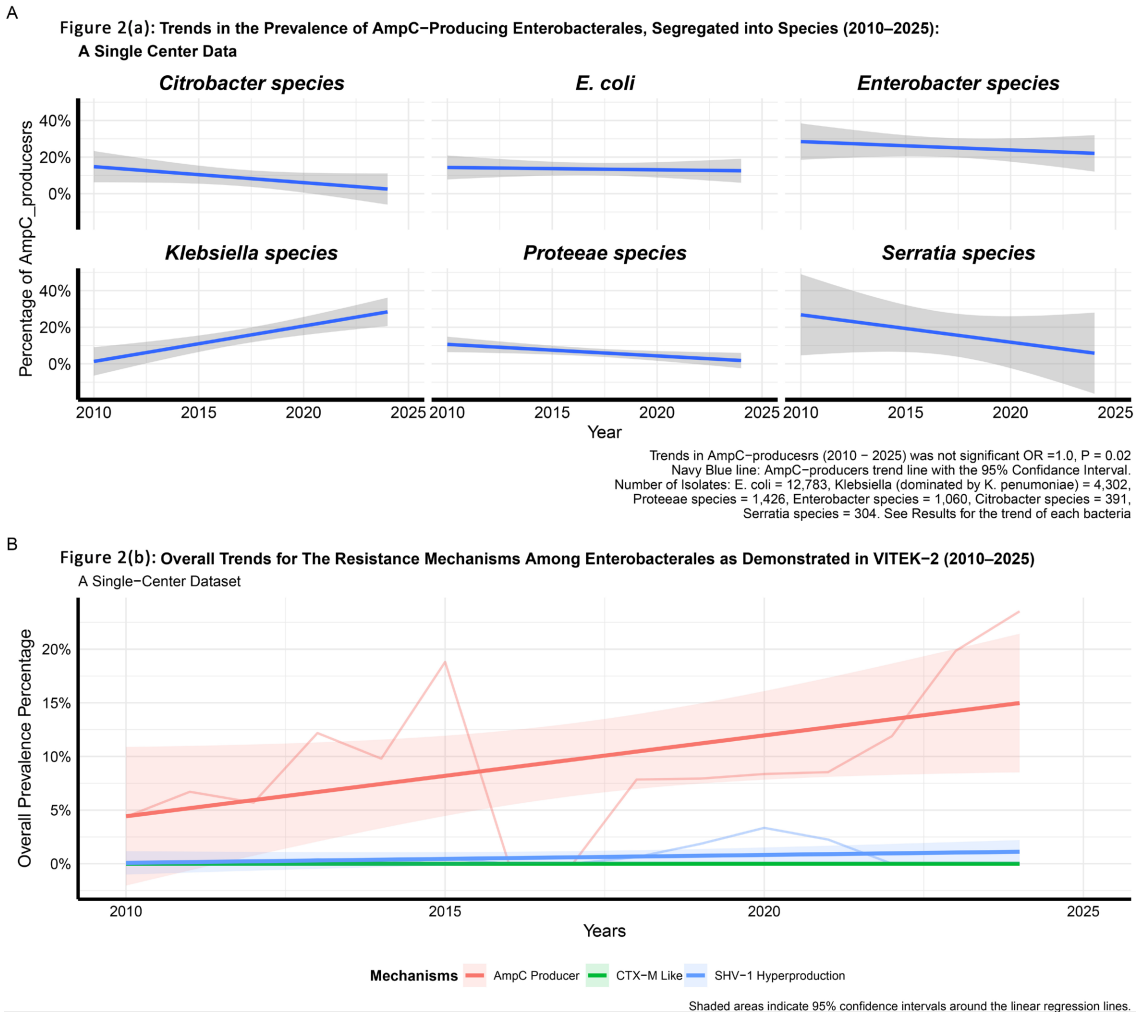

Figure 2. (a) Trends in the prevalence of AmpC-producing *Enterobacterales*, segregated into species (2010-2025): A single center data; (b) Overall trends for the resistance mechanisms among *Enterobacterales* as demonstrated in VITEK-2 (2010-2025): A single-center dataset.

3.4. SHV-1 Hyperproduction

SHV-1 hyperproduction, an advanced β-lactamase expression profile capable of compromising ceftazidime susceptibility was identified in 8.85% of the total cohort. This phenotype was concentrated in *E. coli* 13.0% and *Klebsiella* species 1.9% (Table 1). Long-term longitudinal surveillance showed that SHV-1 hyperproduction rates remained highly stable and constant across the multi-year study period (OR = 1.04, SE = 0.006, P < 0.000; Figure 2(b)).

3.5. Carbapenem-Resistant Enterobacterales (CRE) and Mechanisms of Resistance

The overall prevalence of the CRE phenotypes was 22.4%. Phenotypic carbapenemase production was most frequent among *Klebsiella* species 25% and *Enterobacter* species 11.0%, with lower rates observed in *Citrobacter* species 4.9%, *Serratia* species 5.3%, *E. coli* 3.0%, and Proteeae 2.3% (Table 1).

Longitudinally, CRE rates exhibited a slow, continuous upward trajectory over the 16-year period, though the annual rate of increase was not clinically significant (Figure 3). This steady chronological baseline was consistently reflected across all evaluated taxa: *E. coli* (OR = 0.998, SE < 0.000, P = 0.0). *Klebsiella* (OR = 1.00, SE < 0.000, P < 0.000). *Enterobacter* (OR = 0.999, SE < 0.000, P < 0.000). Proteeae (OR = 0.998, SE < 0.000, P < 0.000). *Serratia* (OR = 0.999, SE < 0.001, P < 0.000). *Citrobacter* (OR = 0.999, SE < 0.001, P < 0.001).

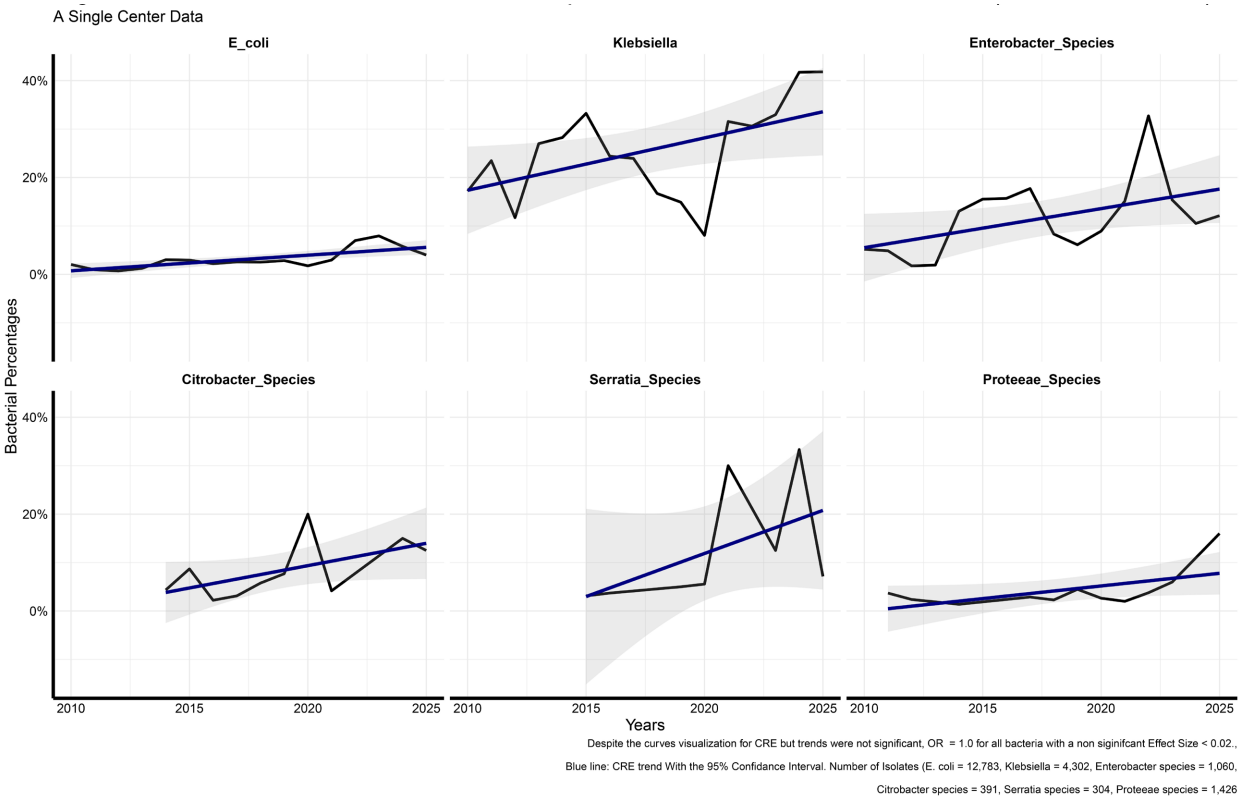

Figure 3. Trends in the prevalence of carbapenem-resistant *Enterobacterales* (CRE; 2010-2025).

To delineate the specific drivers of carbapenem resistance, individual mechanisms were evaluated. Porin impermeability was the most common mechanism, occurring at a total rate of 7.4% across the cohort, driven primarily by *Klebsiella* 20.3%, *Enterobacter* 10.1%, and *E. coli* 2.5%. The cumulative rate for metallo-*β*-lactamases (M*β*Ls) or *Klebsiella pneumoniae* carbapenemase (KPC) production was 1.11%, found in 2.4% of *Klebsiella,* 0.9% of *Enterobacter*, and < 0.5% in both *E. coli* and *Citrobacter*. The inhibitor-resistant TEM or oxacillinase (IRT/OXA) phenotype reached an overall rate of 11.1%, distributed across *E. coli* 16.0%, *Klebsiella* 17.0%, and Proteeae 11.0%, while Enterobacter, Serratia and Citrobacter 0.0% (Table 1).

3.6. Phenotypic Antimicrobial Susceptibility Profiles

Antimicrobial susceptibility profiles were stratified by specific VITEK® 2-defined phenotypic resistance mechanisms. Isolates sharing identical, discrete resistance mechanisms were grouped together to evaluate susceptibility against antimicrobial agents commonly utilized in clinical practice either as primary definitive therapies or as synergistic components.

For **ESBL-PE** isolates, overall susceptibility rates ranged from 37.0% to 45.0%. The highest susceptibility rates were observed for aminoglycosides, fluoroquinolones, piperacillin-tazobactam, and tigecycline, whereas the lowest susceptibility rates were noted for aztreonam 37.0% and minocycline 37.0%.

Among **AmpC-producing** strains, susceptibility rates across all tested antimicrobials were notably lower, ranging from 8.8% to 24.0%. Susceptibility peaked for ceftazidime-avibactam 24.0%, ceftolozane-tazobactam 24.0%, meropenem-vaborbactam 23.0%. Minocycline 13.0%, aztreonam 14.0%. Aminoglycosides, fluoroquinolones, piperacillin-tazobactam, and tigecycline ranged from 8.8% to 12.0%.

For **SHV-1 hyperproducers**, susceptibility to aminoglycosides, quinolones, and piperacillin -tazobactam ranged from 7.0% to 7.9%. Susceptibility rates for tigecycline, aztreonam, and minocycline clustered between 3.1% and 5.7%.

The collective CRE phenotype demonstrated susceptibility rates for minocycline 34.0%, ceftazidime-avibactam 31.0%, and 32% for ceftolozane/tazobactam, aztreonam was 31.0%, while Aminoglycosides, quinolones, and tigecycline exhibited susceptibilities 12.0% to 18% with tigecycline on the high side. Isolates displaying **M*β*L or KPC** phenotypes showed profound multidrug resistance, with susceptibility rates across all tested clinical agents remaining ≤ 0.8%. Strains expressing the **TEM or OXA (IRT)** phenotype exhibited susceptibility rates ranging from 12.0% to 16.0%, with tigecycline demonstrating the highest activity. Susceptibilities to ceftazidime-avibactam and ceftolozane-tazobactam were remarkably suppressed at 3.5% and 3.6%, respectively.

For isolates whose resistance was mediated by **porin impermeability**, susceptibility was 30% for aztreonam and minocycline, 23% for meropenem-vaborbactam, Ceftazidime-avibactam 12%, ceftolozane-tazobactam13%, piperacillin-

tazobactam 11%, the remaining agents were uniformly suppressed ≤ 10.0%. Finally, evaluating the specific **carbapenemase enzyme** subgroup revealed susceptibility rates of 90% for meropenem-vaborbactam, 32.0% for both ceftazidime/avibactam and ceftolozane/tazobactam, minocycline 18%, aztreonam 16%. Susceptibility dropped to 12.0% for tigecycline, 11.0% for piperacillin-tazobactam, and 8.5% to 11.0% for aminoglycosides and quinolones (Table 2).

Table 2. Counts and percentages of multidrug-resistant (MDR) *Enterobacterales* across identified resistance mechanisms and their susceptibility to selected antimicrobial agents*.

Identified mechanisms	AMK N = 21,432[1]	AZT N = 2,913[1]	CAZ-AVI N = 854[1]	CEFT-TAZ N = 824[1]	CIP N = 24,464[1]	GENT N = 26,363[1]	LEV N = 20,648[1]	MER-VAB N = 30[1]	MIN N = 3,156[1]	PIP-TAZ N =22,612[1]	TIG N = 18,772[1]
ESBL#	5,728 (45%)	145 (37%)	0 (NA%)	0 (NA%)	5,899 (45%)	5,898 (45%)	5,077 (45%)	0 (NA%)	145 (37%)	5,538 (45%)	4,111 (44%)
CTX-M LIKE	631 (2.9%)	288 (9.9%)	12 (1.4%)	12 (1.5%)	670 (2.7%)	671 (2.5%)	596 (2.9%)	0 (0%)	287 (9.1%)	631 (2.8%)	478 (2.5%)
AmpC producers	2,533 (12%)	408 (14%)	202 (24%)	200 (24%)	2,675 (11%)	2,675 (10%)	1,812 (8.8%)	7 (23%)	400 (13%)	2,552 (11%)	1,789 (9.5%)
SHV1 Hyperproduction	1,700 (7.9%)	100 (3.4%)	0 (0%)	0 (0%)	1,705 (7.0%)	1,704 (6.5%)	1,615 (7.8%)	0 (0%)	99 (3.1%)	1,621 (7.2%)	1,063 (5.7%)
CRE#	2,575 (12%)	875 (31%)	255 (31%)	252 (32%)	3,525 (15%)	3,559 (15%)	2,677 (16%)	0 (NA%)	1,026 (34%)	3,430 (15%)	2,726 (18%)
MβL or KPC	86 (0.4%)	23 (0.8%)	0 (0%)	0 (0%)	121 (0.5%)	120 (0.5%)	119 (0.6%)	0 (0%)	23 (0.7%)	99 (0.4%)	50 (0.3%)
IRT (TEM) OR OXA	2,924 (14%)	424 (15%)	30 (3.5%)	30 (3.6%)	3,249 (13%)	3,250 (12%)	3,136 (15%)	0 (0%)	447 (14%)	3,208 (14%)	2,975 (16%)
Impermeability	2,040 (9.5%)	880 (30%)	104 (12%)	104 (13%)	2,445 (10.0%)	2,438 (9.2%)	1,798 (8.7%)	7 (23%)	948 (30%)	2,390 (11%)	1,618 (8.6%)
Carbapenemase	1,832 (8.5%)	460 (16%)	271 (32%)	267 (32%)	2,566 (10%)	2,616 (9.9%)	2,179 (11%)	27 (90%)	582 (18%)	2,557 (11%)	2,188 (12%)

Note: colistin was not include, it is not approved to be tested by VITEK 2, **#:** All isolates data, and tested to carbapenem (Imipenem, Ertapenem, Meropenem), **CRE:** Carbapenem resistant *Enterobacterales*, **1:** n (%), **AMK:** Amikacin, **AZT:** Aztreonam, **CAZ-AVI:** Ceftazidim-Avibactam, **CEFT-TAZ:** Ceftolozon-Tazobactam, **CIP:** Ciprofloxacin, **GENT:** Gentamycin, **LEV:** Levofloxacin, **MER-VAB:** Meropenem-Vaborbactam, **MIN:** Minocycline, **PIP-TAZ:** Piperacillin-Tazobactam, **TIG:** Tigecycline, **IRT (TEM) or OXA:** inhibitor resistance or oxacillinase, **MβL or KPC:** Metallo-β-lactamase or *K. pneumoniae* carbapenemase, **NA**: not tested.

4. Discussion

The relentless escalation of antimicrobial resistance (AMR) among Gram-negative bacilli is a paramount public health priority, as underscored by comprehensive surveillance frameworks from the World Health Organization Global Antimicrobial Resistance and Use Surveillance System (GLASS) and the United States Centers for Disease Control and Prevention (CDC) [14] [15]. Within the family *Enterobacterales*, the dissemination of complex resistance mechanisms is driven by a potent combination of clonal expansion and horizontal gene transfer facilitated by mobile genetic elements [16].

A profound epidemiological divergence exists between global economic regions. High-income countries (HICs) have successfully controlled or mitigated

the trajectory of AMR through the rigorous enforcement of restricted antimicrobial stewardship programs (ASPs) and active infection prevention and control (IPC) infrastructure. Conversely, low- and middle-income countries (LMICs) continue to experience a surge in resistance metrics. This trajectory is fueled by high baseline rates of unnecessary antimicrobial use (UAU), unrestricted access to broad-spectrum agents, fragmented or under-resourced stewardship frameworks, and partially implemented IPC programs [17]-[19].

This systemic vulnerability is reflected in global meta-analyses, which demonstrate a massive burden of extended-spectrum β-lactamase-producing *Enterobacterales* (ESBL-PE) across LMICs with reported hospital prevalence rates reaching 40% in Pakistan, 49% in Ethiopia, 34.6% in Nigeria, 29% in Nepal, and 42% across East African healthcare centers. In sharp contrast, baseline prevalence rates in HICs consistently remain below 10% [18] [20]-[23].

The cumulative ESBL-PE prevalence in our cohort was approximately 29.8%, demonstrating a high degree of regional alignment with other LMIC tertiary centers. Genus-specific stratification revealed clear phenotypic disparities: ESBL expression was highly concentrated in *Escherichia coli* 47% and *Klebsiella pneumoniae* 36%, the rest of *Enterobacterales* had the phenotype ($ESBL_{CTX\text{-}M\ like}$) which was recorded in Proteeae, *Serratia* species, *Citrobacter* species, and Enterobacter at 5.3% to 1.7%. This may point to a recognized diagnostic limitation of automated systems. In wild-type species expressing inducible, chromosomally encoded β-lactamases (such as AmpC), hyper-expression can phenotypically mask co-existing plasmid-mediated ESBLs (such as $bla_{CTX\text{-}M}$), creating a critical diagnostic blind spot during VITEK® 2 automated expert system (AES) profiling. Crucially, our 16-year longitudinal analysis revealed that despite visual fluctuations indicating a downward trend, the overall annual prevalence of ESBL-PE remained statistically stagnant without meaningful clinical shifts (OR = 0.968, SE = 0.006, $P < 0.001$).

AmpC β-lactamase production was identified in 13.9% of our total *Enterobacterales* cohort, led by *Enterobacter* species at 25%, *Klebsiella* species at 18% and *E. coli* 13.0%. This significantly exceeds baseline limits observed in HICs, where AmpC expression among *E. coli* and *K. pneumoniae* ranges from 5% to 10%. Our figures fall midway between HIC and the data reported from South Asian and East African tertiary environments (19% to 23%), and they remain far from the extreme values > 30% documented in parts of India and Egypt [21]. A major diagnostic challenge in resource-limited settings is the inability to routinely differentiate between chromosomal de-repression and plasmid-mediated AmpC production. The latter is epidemiologically concerning because these plasmids frequently act as vectors for the co-transfer of auxiliary resistance elements, including ESBLs and aminoglycoside-modifying enzymes.

Furthermore, in our dataset, *Enterobacter* and *Serratia* isolates displayed high rates of AmpC expression alongside nearly undetectable ESBL phenotypes. This pattern strongly indicates that a substantial proportion of these isolates were dual-producers (AmpC + ESBL); the VITEK® 2 AES algorithm systematically occasion-

ally prioritize reporting the dominant, clinically definitive AmpC phenotype to guide therapy. Longitudinally, the cumulative AmpC baseline (**Figure 2**) remained static over the 16-year period (OR = 1.04, SE = 0.006, P < 0.000), driven by a slow upward trajectory in *Klebsiella* species (OR = 1.08, SE = 0.01, P < 0.000) balanced against a steady decline in *Serratia* species (OR = 0.881, SE = 0.037, P = 0.000). SHV1-hyperproduction rate is 8.85%, which is a concerning resistance mechanism for it is capable of compromising ceftazidime susceptibility, and it is concentrated in *E. coli* (13.0%) and *Klebsiella* species (1.9%), both are abundant bacteria frequently cause clinical infections. However, a relatively promising that its trend remains almost unchanged over the years (OR = 1.04, SE = 0.006, P < 0.000) [24] [25].

Our carbapenem-resistant *Enterobacterales* (CRE) totaled 22.4%, they pose the most critical clinical threat within this family, carrying an attributable mortality rate of 40% to 50% in patients who develop overt sepsis. Furthermore, active surveillance highlights the consequences of colonization; patients screening positive for CRE via perirectal swabs within the first week of intensive care unit (ICU) admission face a 27% baseline risk of subsequent all-cause mortality [26] [27].

The therapeutic implications of our susceptibility data are deeply concerning. The aggregate susceptibility of CRE isolates to novel, premier β-lactam/β-lactamase inhibitor combinations, specifically ceftazidime-avibactam was 31.0% and ceftolozane-tazobactam was 32.0%. When stratified by specific sub-mechanisms, both agents maintained moderate activity against classic carbapenemase-producing strains (32% susceptibility). When tested against isolates characterized by porin impermeability their susceptibility was 12.0% and 13.0% respectively, However, when deployed against inhibitor-resistant TEM/OXA formulations, or metallo-β-lactamase (MβL/KPC) expression, susceptibility rates dropped precipitously below 5%.

These values provide a valuable clinical counterpoint to smaller, targeted molecular evaluations in regional literature. For example, Alatoom *et al.* evaluated 120 characterized ESBL and CRE strains using Etest minimum inhibitory concentration (MIC) strips and corroborative PCR assays. Their investigation reported that 100% of pure ESBL-PE isolates were susceptible to ceftazidime-avibactam, whereas our aggregate clinical database lacked historic susceptibility metrics for these specific agents during the early phases of the study period, primarily due to localized formulary restrictions and the delayed deployment of updated automated AST cards. Furthermore, Alatoom *et al.* observed that 45% of CRE isolates were susceptible to ceftazidime-avibactam compared to just 10% for ceftolozane-tazobactam. While our unstratified CRE cohort showed identical baseline susceptibilities (32% for both agents), our molecularly categorized subgroups revealed crucial mechanistic boundaries. Just as Alatoom *et al.* noted that the presence of the New Delhi metallo-β-lactamase (bla_{NDM-1}) completely abolished the clinical utility of ceftazidime-avibactam while isolates carrying oxacillinase-48 (bla_{OXA-48}) variants remained highly susceptible, our clinical cohort demonstrated a sup-

pressed susceptibility rate of 3.5% within the TEM/OXA. This underscores the absolute necessity of integrating rapid phenotypic or molecular sub-mechanism screening to successfully navigate the narrowed therapeutic options available for these pathogens [28].

As alluded to earlier, our study has limitations. First, it is a single-center study. Second, while the phenotypic resistance patterns described here aligned with the VITEK 2 Advanced Expert System (AES) and carried a decent probability percentage, they lack the precision of molecular methods in detecting exact genotypic resistance mechanisms. However, this probability data remains highly adequate for clinical infectious disease management when paired with antimicrobial susceptibility testing (AST) interpretation, even though AST criteria are subject to change by the CLSI or EUCAST. Another limitation is that newer antimicrobial agents—specifically ceftazidime-avibactam, ceftolozane-tazobactam, and meropenem-vaborbactam—were introduced late in the study period. This late introduction precluded accurate long-term trend calculations and year-over-year comparisons.

5. Conclusions

This 16-year longitudinal analysis establishes that while multi-drug resistant *Enterobacterales* phenotypes have not experienced a significant exponential increase since 2010, they have successfully consolidated their presence at an elevated, highly stable baseline within our clinical environment. Generalized linear modeling confirmed that chronological shifts over the 16-year study period lacked clinical significance (OR = 0.998 - 0.999, $P < 0.001$), representing a negligible annual change of less than 0.2% across all primary species (*E. coli*, *Klebsiella*, *Enterobacter*, Proteeae, *Serratia*, and *Citrobacter*).

The profound epidemiological disparity in baseline resistance rates between HICs and LMICs highlights systemic challenges, including excessive levels of unnecessary antibiotic use, rapidly switching treatment courses, and empirical overprescription. Although the stabilization of these resistance patterns indicates that current infection control and containment protocols have successfully prevented further exponential compounding, the lack of a meaningful long-term reduction indicates that existing interventions are insufficient to reverse the endemic status of these pathogens. Reversing these entrenched resistance baselines will require a structural transition from passive containment to aggressive, strictly enforced, and multi-disciplinary antimicrobial stewardship and surveillance.

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Conflicts of Interest

The author declares no conflicts of interest regarding the publication of this paper.

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