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DISTRIBUTION OF BACTERIAL ISOLATES AND ESBL PRODUCTION AMONG SELECTED ENTEROBACTERALES FROM LOWER RESPIRATORY TRACT SPECIMENS AT A TERTIARY HOSPITAL IN VIETNAM

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ABSTRACT

Lower respiratory tract infections(LRTIs) are commonly associated with bacterial pathogens, and local microbiological data may support antimicrobial management. This descriptive cross-sectional study aimed to describe bacterial isolates from lower respiratory tract specimens and determine extended-spectrum beta-lactamase (ESBL) production among selected Enterobacterales at Can Tho General Hospital, Vietnam, from June 2025 to June 2026. Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system. Among 635 isolates representing 26 species, Gram-negative bacteria accounted for 97.3%. Patients aged ≥61 years accounted for 78.4% of isolates and 72.6% were from the Intensive Care Unit. The predominant species were Klebsiella pneumoniae (35.9%), Acinetobacter baumannii (22.4%), Klebsiella aerogenes (12.3%), Pseudomonas aeruginosa (10.6%), and Escherichia coli (9.4%), together accounting for 90.6% of isolates. Among 295 K. pneumoniae, E. coli, and Proteus mirabilis isolates evaluated, 59.7% exhibited an ESBL phenotype. ESBL positivity was 60.5%, 56.7%, and 57.1%, respectively. These findings provide local data on bacterial distribution and ESBL production that may support microbiological surveillance and antimicrobial stewardship.

Keywords: Lower respiratory tract infections; Gram-negative bacteria; Enterobacterales; ESBL; antimicrobial resistance.

INTRODUCTION

Lower respiratory tract infectionsare common causes of hospitalization and remain an important clinical concern, particularly among older adults and critically ill patients. In hospital settings, timely identification of bacterial pathogens and appropriate antimicrobial therapy are important for effective management of these infections (Kalil *et al.,* 2016; Wan *et al.,* 2025).

Gram-negative bacteria are frequently isolated from lower respiratory tract specimens, with *Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa,* and *Escherichia coli* among the clinically relevant pathogens reported in hospitalized patients. Several of these organisms are also important because of their ability to acquire multiple antimicrobial resistance mechanisms (Santajit & Indrawattana, 2016; World Health Organization, 2024; Singh *et al.,* 2025).

Among Enterobacterales, extended-spectrum beta-lactamase (ESBL) production is an important mechanism of resistance to extended-spectrum cephalosporins and aztreonam and may limit available antimicrobial treatment options. Laboratory detection and local surveillance of ESBL-producing isolates are therefore useful for supporting antimicrobial selection and stewardship (Poirel *et al.,* 2018; Tamma *et al.,* 2024; CLSI, 2026).

Studies in Vietnam have reported a predominance of Gram-negative bacteria in lower respiratory tract specimens, although bacterial distribution and antimicrobial susceptibility may vary among hospitals and over time (Hua *et al.,* 2022; Dung *et al.,* 2025).

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Therefore, this study aimed to describe the distribution of bacterial isolates recovered from lower respiratory tract specimens and to determine the prevalence of ESBL production among selected Enterobacterales isolates at a tertiary hospital in Vietnam.

MATERIAL AND METHODS

Materials: The study population comprised patients with clinically diagnosed lower respiratory tract infections who had lower respiratory tract specimens submitted for bacterial culture at Can Tho General Hospital between June 2025 and June 2026. Specimens included sputum, endotracheal aspirates, and bronchial or bronchoalveolar lavage fluid.

Inclusion criteria: Patients with a clinical diagnosis of LRTI and an eligible lower respiratory tract specimen yielding at least one bacterial isolate were included. Only the first eligible bacterial isolate from each patient admission was included in the analysis. Patients were required to have sufficient demographic and clinical information for analysis.

Exclusion criteria: Specimens were excluded if they were considered contaminated, were processed more than 24 hours after collection, or were positive for *Mycobacterium tuberculosis*. Repeated isolates from the same patient during the same admission were excluded.

Study design and sampling method: A descriptive cross-sectional study was conducted at Can Tho General Hospital from June 2025 to June 2026. Consecutive sampling was applied throughout the study period. All eligible patients meeting the inclusion criteria during the study period were consecutively included.

Research contents: Demographic and clinical variables included sex, age group (<41, 41–60, and ≥61 years), hospital unit, and year of isolation. Microbiological variables included Gram-stain group, bacterial species, and distribution of the major bacterial pathogens. ESBL phenotype was evaluated among *Escherichia coli, Klebsiella pneumoniae,* and *Proteus mirabilis* isolates.

Data collection and statistical analysis:

Lower respiratory tract specimens, including sputum, endotracheal aspirates, and bronchial or bronchoalveolar lavage fluid, were collected and transported to the microbiology laboratory according to the hospital's standard procedures. Sputum specimens were assessed microscopically for specimen quality before culture. Eligible specimens were cultured using routine microbiological procedures. Bacterial identification and antimicrobial susceptibility testing were performed using the VITEK 2 Compact system (bioMérieux, France) (bioMérieux, 2026).

ESBL phenotype analysis was restricted to *E. coli, K. pneumoniae,* and *P. mirabilis*. Non-fermenting Gram-negative bacteria, including *Pseudomonas, Acinetobacter, Stenotrophomonas,* and *Burkholderia* species, were not included in the ESBL analysis. ESBL detection and antimicrobial susceptibility results were interpreted according to validated laboratory criteria and relevant antimicrobial susceptibility testing standards (Spanu *et al.,* 2006; EUCAST, 2017; CLSI, 2026).Data were coded and analyzed using SPSS version 20.0. Categorical variables were summarized as frequencies and percentages. Differences between categorical variables were assessed using the chi-square test or Fisher's exact test, as appropriate. A two-sided *p* value <0.05 was considered statistically significant.

Ethical Considerations

The study was approved by the Ethics Committee of Nam Can Tho University (Approval No. 64/2025/PCT-HĐYĐ, June 1, 2025). Participation was voluntary, and written informed consent was obtained from all participants prior to enrollment. Participant confidentiality was maintained throughout the study, and all collected data were used solely for research purposes.

RESULTS

Characteristics of bacterial isolates and Gram-stain distribution

A total of 635 bacterial isolates were recovered during the study period, including 256 isolates in 2025 and 379 in 2026. The distributions by sex and age group were similar between the two years (p = 1.000 and p = 0.064, respectively). Patients aged ≥61 years accounted for the largest proportion of isolates in both 2025 (75.4%) and 2026 (80.5%). In contrast, the distribution across hospital units differed significantly between the two years (p < 0.001). The ICU accounted for the largest proportion in both years, although its proportion decreased from 80.5% in 2025 to 67.3% in 2026 (Table 1).

Table 1. Characteristics of bacterial isolates by year

Characteristic	Category	2025, n (%) n=256	2026, n (%) n=379	*p*-value
Sex	Male	134(52.3)	199 (52.5)	1.000
	Female	122 (47.7)	180 (47.5)	
Age group	<41 years	17 (6.6)	11 (2.9)	0.064
	41–60 years	46 (18.0)	63 (16.6)	
	≥61 years	193 (75.4)	305 (80.5)	
Hospital unit	Intensive care unit	206 (80.5)	255 (67.3)	<0.001
	General internal medicine	33 (12.9)	55 (14.5)	
	Other departments	17 (6.6)	69 (18.2)	

Gram-negative bacteria accounted for 618 of 635 isolates (97.3%), whereas Gram-positive bacteria accounted for 17 (2.7%). Gram-stain distribution did not differ significantly according to sex (p = 0.436) or age group (p = 0.527). However, a significant difference was observed across hospital units (p = 0.020). Gram-negative bacteria accounted for 98.3% of isolates from the ICU, compared with 96.6% from General Internal Medicine and 93.0% from other departments (Table 2).

Table 2. Distribution of Gram-negative and Gram-positive bacterial isolates

Characteristic	Category	Gram-negative n (%)	Gram-positive n (%)	*p*-value
Sex	Male	322 (96.7)	11 (3.3)	0.436
	Female	296 (98.0)	6 (2.0)	
Age group	<41 years	28 (100.0)	0 (0.0)	0.527
	41–60 years	107 (98.2)	2 (1.8)	
	≥61 years	483 (97.0)	15 (3.0)	
Hospital unit	Intensive Care Unit	453 (98.3)	8 (1.7)	0.020
	General Internal Medicine	85 (96.6)	3 (3.4)	
	Other departments	80 (93.0)	6 (7.0)	

Gram-negative bacteria predominated among isolated pathogens as in Table 2, accounting for 97.3% of all lower respiratory tract isolates, whereas Gram-positive bacteria comprised only 2.7%. No statistically significant differences in Gram stain distribution were observed across sex (p = 0.436) or age groups (p = 0.527). However, a significant association was observed for hospital units (*p* = 0.020), with Gram-negative isolates being the most common in the Intensive Care Unit (98.3%, n = 453), while other departments showed a slightly higher proportion of Gram-positive organisms (7.0%).

Bacterial species distribution and temporal patterns

A total of 26 bacterial species were identified from lower respiratory tract specimens. *Klebsiella pneumoniae* was the most frequently isolated species (228/635, 35.9%), followed by *Acinetobacter baumannii* (142/635, 22.4%), *Klebsiella aerogenes* (78/635, 12.3%), *Pseudomonas aeruginosa* (67/635, 10.6%), and *Escherichia coli* (60/635, 9.4%). Together, these five species accounted for 90.6% of all bacterial isolates. Among Gram-positive organisms, *Staphylococcus aureus* was the most frequently identified species (9/635, 1.4%) (Table 3).

Table 3. Distribution of bacterial species isolated from lower respiratory tract specimens

Gram-stain category	Species	2025, n n=256	2026, n n=379	Overall n	Overall %
Negative	*Klebsiella pneumonia*	90	138	228	35.9
Negative	*Acinetobacter baumannii*	66	76	142	22.4
Negative	*Klebsiella aerogenes*	28	50	78	12.3
Negative	*Pseudomonas aeruginosa*	28	39	67	10.6

Negative	*Escherichia coli*	28	32	60	9.4
Negative	*Enterobacter cloacae complex*	4	6	10	1.6
Negative	*Stenotrophomonas maltophilia*	1	9	10	1.6
Positive	*Staphylococcus aureus*	4	5	9	1.4
Negative	*Proteus mirabilis*	0	7	7	1.1
Negative	*Achromobacter xylosoxidans*	0	3	3	0.5
Positive	*Rothia kristinae*	1	2	3	0.5
Negative	*Acinetobacter lwoffii*	0	2	2	0.3
Negative	*Serratia marcescens*	0	2	2	0.3
Positive	*Staphylococcus haemolyticus*	1	1	2	0.3
Negative	*Acinetobacter haemolyticus*	0	1	1	0.2
Negative	*Burkholderia cepacia group*	0	1	1	0.2
Negative	*Citrobacter koseri*	1	0	1	0.2
Positive	*Enterococcus faecalis*	1	0	1	0.2
Positive	*Kocuria kristinae*	1	0	1	0.2
Negative	*Morganella morganii*	1	0	1	0.2
Negative	*Proteus vulgaris*	0	1	1	0.2
Negative	*Pseudomonas putida*	0	1	1	0.2
Negative	*Raoultella ornithinolytica*	0	1	1	0.2
Negative	*Serratia fonticola*	0	1	1	0.2
Negative	*Sphingomonas paucimobilis*	0	1	1	0.2
Positive	*Staphylococcus sciuri*	1	0	1	0.2

The proportional distribution of the principal Gram-negative species was generally similar between 2025 and 2026. *K. pneumoniae* remained the predominant species in both years, accounting for 35.2% and 36.4% of isolates, respectively. Although *A. baumannii* decreased from 25.8% in 2025 to 20.1% in 2026 and *Stenotrophomonas maltophilia* increased from 0.4% to 2.4%, these differences were not statistically significant. No significant year-to-year differences were observed for any of the principal Gram-negative species evaluated (all $p > 0.05$) (Table 4).

Table 4. Yearly distribution of the principal Gram-negative bacterial species

Species	2025, n (%) n=256	2026, n (%) n=379	Total, n	*p*-value
Klebsiella pneumoniae	90 (35.2)	138 (36.4)	228	0.746
Acinetobacter baumannii	66 (25.8)	76 (20.1)	142	0.089
Klebsiella aerogenes	28(10.9)	50 (13.2)	78	0.396
Pseudomonas aeruginosa	28 (10.9)	39 (10.3)	67	0.795
Escherichia coli	28 (10.9)	32 (8.4)	60	0.292
Enterobacter cloacae complex	4 (1.6)	6 (1.6)	10	0.984
Stenotrophomonas maltophilia	1 (0.4)	9 (2.4)	10	0.104

ESBL production among selected Enterobacterales

Among the 295 *K. pneumoniae, E. coli,* and *Proteus mirabilis* isolates evaluated for ESBL production, 176 (59.7%) exhibited an ESBL phenotype. The proportion of ESBL-positive isolates was 60.5% (138/228) for *K. pneumoniae*, 56.7% (34/60) for *E. coli*, and 57.1% (4/7) for *P. mirabilis* (Table 5).

Table 5. ESBL production among Enterobacterales isolates

Species	ESBL-positive n (%)	ESBL-negative n (%)	Total
Klebsiella pneumoniae	138 (60.5)	90 (39.5)	228
Escherichia coli	34 (56.7)	26 (43.3)	60
Proteus mirabilis	4 (57.1)	3 (42.9)	7
Overall	**176 (59.7)**	**119 (40.3)**	**295**

Among 295 isolates evaluated for ESBL production, 176 (59.7%) exhibited an ESBL phenotype. ESBL positivity was highest in *K. pneumoniae* (60.5%), followed by *P. mirabilis* (57.1%) and *E. coli* (56.7%).

DISCUSSION

This study described the distribution of bacterial isolates recovered from lower respiratory tract specimens at a tertiary hospital in Vietnam. Gram-negative bacteria accounted for 97.3% of the 635 isolates, and the five most frequently identified species were *Klebsiella pneumoniae, Acinetobacter baumannii, Klebsiella aerogenes, Pseudomonas aeruginosa,* and *Escherichia coli*. Among the selected Enterobacterales evaluated for ESBL production, 59.7% exhibited an ESBL phenotype.

The predominance of Gram-negative bacteria observed in this study is generally consistent with previous reports of bacterial pathogens isolated from lower respiratory tract specimens. Studies conducted in Vietnam have also reported Gram-negative organisms, including *K. pneumoniae, A. baumannii, P. aeruginosa,* and *E. coli*, among commonly identified respiratory isolates (Hua *et al.,* 2022; Dung *et al.,* 2025). Similar bacterial profiles have been reported in other hospital settings, although the relative distribution of individual species varies across studies (Singh *et al.,* 2025). Such variation may reflect differences in patient populations, hospital settings, specimen types, and local epidemiological characteristics (Santajit & Indrawattana, 2016; World Health Organization, 2024).

Klebsiella pneumoniae was the most frequently identified species in the present study, accounting for 35.9% of all isolates, followed by *A. baumannii* (22.4%), *K. aerogenes* (12.3%), *P. aeruginosa* (10.6%), and *E. coli* (9.4%). Together, these species accounted for 90.6% of the bacterial isolates. Several of these Gram-negative organisms are commonly encountered in healthcare settings and possess multiple mechanisms that may facilitate antimicrobial resistance and persistence. Biofilm formation has also been described in clinical *K. pneumoniae* isolates (Holmes *et al.,* 2021; Nirwati *et al.,* 2019). However, biofilm formation and specific resistance mechanisms were not investigated in the present study.

Most isolates were recovered from patients aged ≥61 years (78.4%) and from those admitted to the ICU (72.6%). Gram-negative bacteria represented 98.3% of ICU isolates, and Gram-stain distribution differed significantly among hospital units ($p = 0.020$). Hospital-acquired and ventilator-associated respiratory infections are commonly encountered in critically ill populations, in whom microbiological evaluation is important for guiding treatment (Kalil *et al.,* 2016). Nevertheless, the present study did not evaluate mechanical ventilation, previous antimicrobial exposure, length of hospital stay, or other potential risk factors. Therefore, the observed distribution should be interpreted descriptively rather than as evidence that ICU admission or older age was independently associated with particular bacterial pathogens.

The proportions of the principal Gram-negative species did not differ significantly between 2025 and 2026. *K. pneumoniae* remained the most frequent species in both years, while the observed changes in *A. baumannii* and *Stenotrophomonas maltophilia* did not reach statistical significance. These results suggest a generally similar distribution of the principal bacterial species during the study period. Periodic local surveillance may be useful because bacterial distributions and antimicrobial susceptibility patterns can vary between settings and over time (Wan *et al.,* 2025; Dung *et al.,* 2025). Among the 295 *K. pneumoniae, E. coli,* and *Proteus mirabilis* isolates evaluated for ESBL production, 176 (59.7%) exhibited an ESBL phenotype. The proportions were relatively similar among *K. pneumoniae* (60.5%), *P. mirabilis* (57.1%), and *E. coli* (56.7%). ESBL production is a recognized mechanism of resistance to extended-spectrum cephalosporins and aztreonam among Enterobacterales and may coexist with resistance to other antimicrobial classes (Poirel *et al.,* 2018; Tamma *et al.,* 2024). These findings provide local information on the frequency of ESBL phenotypes among the selected Enterobacterales species examined in this study.

ESBL results should be interpreted within the scope of the laboratory methods used. ESBL evaluation was restricted to *K. pneumoniae, E. coli,* and *P. mirabilis* and did not include non-fermenting Gram-negative bacteria such as *Pseudomonas* and *Acinetobacter*. Therefore, the ESBL proportion of 59.7% applies only to the 295 selected Enterobacterales isolates rather than to all Gram-negative isolates. Phenotypic ESBL detection and antimicrobial susceptibility testing require standardized laboratory procedures and interpretation criteria (Spanu *et al.,* 2006; EUCAST, 2017; CLSI, 2026). In the present study, bacterial identification and antimicrobial susceptibility testing were performed using the automated VITEK 2 Compact system (bioMérieux, 2026).

The observed bacterial distribution and ESBL phenotype frequencies may provide useful local information for microbiological surveillance and antimicrobial stewardship. However, these findings alone cannot determine the most appropriate empirical antimicrobial regimen. Treatment decisions should also consider the site and severity of infection, individual susceptibility results, previous antimicrobial exposure, and patient-specific clinical factors (Tamma *et al.,* 2024).

This study has several limitations. It was conducted at a single hospital, which may limit the generalizability of the findings to other healthcare settings. The analysis was based on cultured bacterial isolates and did not include viral, fungal, or atypical respiratory pathogens. Clinical factors potentially related to bacterial distribution and antimicrobial resistance were not analyzed. In addition, ESBL evaluation was limited to three Enterobacterales species and did not include molecular characterization of ESBL-associated genes. Despite these limitations, the study provides local descriptive data on bacterial isolates recovered from lower respiratory tract specimens and ESBL phenotypes among selected Enterobacterales.

CONCLUSION

Gram-negative bacteria predominated among lower respiratory tract isolates at the study hospital, accounting for 97.3% of all isolates. *Klebsiella pneumoniae, Acinetobacter baumannii, Klebsiella aerogenes, Pseudomonas aeruginosa,* and *Escherichia coli* were the most frequently identified species, together accounting for 90.6% of isolates. Among the 295 *K. pneumoniae, E. coli,* and *Proteus mirabilis* isolates evaluated for ESBL production, 59.7% exhibited an ESBL phenotype. These findings provide local microbiological data that may support antimicrobial stewardship and the selection of appropriate empirical therapy for lower respiratory tract infections.

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