

Distribution of gram-negative bacteria in lower respiratory tract infections and their susceptibility patterns to beta-lactam antibiotics during the COVID-19 pandemic

Conny Riana Tjampakasari^{1*}, Reyhan Afif Suchahyo², Dimas Seto Prasetyo¹

¹Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

²Department of Medical Education, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia

Received: May 2026, Accepted: August 2026

ABSTRACT

Background and Objectives: Inappropriate antibiotic use can lead to resistance, particularly in Gram-negative bacteria. The aim of this study was to identify the distribution of Gram-negative bacteria responsible for lower respiratory tract infections and to assess their susceptibility profiles to beta-lactam antibiotics in the periods before and during the COVID-19 pandemic.

Materials and Methods: This retrospective cross-sectional study utilized secondary data from WHONET 2022, drawing on a database of Gram-negative bacteria isolated from lower respiratory tract specimens processed at the Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia, during the period of 2018-2021. Susceptibility to ampicillin-sulbactam, ceftazidime, cefepime, meropenem, and piperacillin-tazobactam was assessed. Comparisons between the pre-pandemic and pandemic periods were conducted using the chi-square test.

Results: A total of 569 samples were analyzed, comprising 383 samples from the pre-pandemic period (2018-2019) and 186 samples from the COVID-19 pandemic period (2020-2021). Twenty-nine Gram-negative bacterial species were identified before the pandemic, compared with 19 species during the COVID-19 pandemic. *Klebsiella pneumoniae* emerged as the most common pathogen, with a prevalence of 30.29% before and 27.96% during the COVID-19 pandemic. Statistical analysis revealed significant differences in the resistance patterns of *Acinetobacter baumannii* to cefepime ($p = 0.036$), ceftazidime ($p = 0.018$), and piperacillin-tazobactam ($p = 0.002$). Conversely, no significant differences were observed for *K. pneumoniae* and *Pseudomonas aeruginosa* ($p > 0.05$).

Conclusion: *K. Pneumoniae* remains the predominant Gram-negative pathogen causing lower respiratory tract infections, whereas *A. baumannii* shows an increased disease burden and resistance during the COVID-19 pandemic. This underscores the importance of continuous surveillance of antimicrobial resistance.

Keywords: Gram-negative bacteria; Beta-lactams; Bacterial distribution; Lower respiratory tract infections; Susceptibility patterns

INTRODUCTION

Coronavirus disease 2019 (COVID-19) has impacted countries across the globe, including Indonesia.

Based on data from the World Health Organization (WHO) as of August 9, 2023, Indonesia has reported over 6.8 million confirmed COVID-19 cases and more than 11,900 deaths (1). COVID-19 predomi-

*Corresponding author: Conny Riana Tjampakasari, Ph.D, Department of Microbiology, Faculty of Medicine, Universitas Indonesia, Jakarta, Indonesia. Tel: 62-21-31922850 Fax: 62-21-3100810 Email: connyrianat@yahoo.com

nantly involves the respiratory tract and is characterized by a wide range of clinical presentations, from asymptomatic infection to severe pneumonia and fatal outcomes (2).

COVID-19 infection can lead to acute injury of lung epithelial cells and damage to the respiratory epithelium, thereby increasing vulnerability to secondary bacterial infections. As a result, empirical antibiotic therapy is commonly administered to hospitalized patients. Nevertheless, excessive or inappropriate antibiotic use may drive the development of antimicrobial resistance, particularly among Gram-negative bacteria responsible for lower respiratory tract infections.

Beta-lactam antibiotics are widely used in the treatment of Gram-negative bacterial infections. However, misuse or inappropriate administration of these agents can lead to the selection of resistant bacterial strains. These antibiotics exert their antibacterial effect by inhibiting bacterial cell wall synthesis through disruption of peptidoglycan formation (3).

The emergence of antimicrobial resistance makes the management of secondary bacterial infections, especially lower respiratory tract infections, increasingly challenging (4).

Data indicate that secondary infections in COVID-19 patients constitute around 18.4% of all recorded COVID-19 cases (5). Although this proportion is relatively low, empirical are administered to approximately 62% of individuals with COVID-19, primarily to prevent or manage suspected secondary bacterial infection (5). This pattern of use raise concerns about the potential for inappropriate antibiotic prescribing and the consequent emergence of antimicrobial resistance (5).

Antimicrobial resistance poses substantial challenges in the management of lower respiratory tract infections and is associated with increased morbidity and mortality. Beta-lactam antibiotics are widely employed in the treatment of Gram-negative bacterial infections. In lower respiratory tract infections such as pneumonia, cephalosporins are commonly administered as first-line agents. In more severe infections caused by Gram-negative pathogens, carbapenems, including meropenem and imipenem, are frequently utilized. An important pathogen linked to severe pneumonia is *Pseudomas aeruginosa* (6).

In 2019, the WHO published a list of priority antibiotic-resistant bacterial pathogens, designating carbapenem-resistant *P. aeruginosa*, carbapenem-

resistant *A. baumannii*, and third-generation cephalosporin-resistant Enterobacterales as organism requiring urgent surveillance (7).

Data on the distribution of Gram-negative bacteria and their susceptibility profiles before and during the COVID-19 pandemic are still scarce in Indonesia. Consequently, this study was conducted to characterize the distribution of Gram-negative bacteria responsible for lower respiratory tract infections and to evaluate their susceptibility patterns to beta-lactam antibiotics in the periods preceding and during the COVID-19 pandemic.

MATERIALS AND METHODS

Study design and ethical approval. This study was conducted as a retrospective descriptive investigation with a cross-sectional design to present data on the distribution of Gram-negative bacteria responsible for lower respiratory tract infections and their susceptibility to beta-lactam antibiotics from the WHONET 2022 software of the Department of Microbiology, Faculty of Medicine, Universitas Indonesia. Ethical approval was granted by the Ethics Committee of the Faculty of Medicine, Universitas Indonesia (No. KET-18/UN2.FI.D1.2/PDP.01/RISET.2025).

Bacterial isolates. A total of 569 isolates collected between January 2018, and December 2021 were included in this study. Clinical isolates were obtained from patients diagnosed with lower respiratory tract infections, and all specimens were processed at the Department of Microbiology, Faculty of Medicine, Universitas Indonesia.

Inclusion criteria. Respiratory specimens collected from patients with clinically diagnosed lower respiratory tract infections during the study period were included if they yielded Gram-negative bacterial growth and had complete bacterial identification and antimicrobial susceptibility testing results available in the WHONET database.

Exclusion criteria. Duplicate isolates from the same patient, isolates with incomplete microbiological or antimicrobial susceptibility data, non-Gram-negative bacterial isolates, and respiratory specimens collected for surveillance or colonization screening without clinical evidence of infection were excluded.

Only isolates from patients who were clinically diagnosed with lower respiratory tract infection were included, to distinguish true infection from colonization. The diagnosis was established by the treating clinician based on clinical manifestations, radiological findings, and microbiological results documented in the hospital information system. Respiratory specimens obtained solely for screening or considered to represent respiratory tract colonization without clinical evidence of infection were excluded from the study.

Bacterial identification. Clinical specimens were cultured on Blood Agar and MacConkey and incubated at 37°C for 18-24 hours. After incubation, colony morphology was evaluated, and Gram staining was performed to verify the presence of Gram-negative, rod-shaped bacteria. Species identification and antimicrobial susceptibility profiling had been previously established using the VITEK® 2 Compact automated system (bioMérieux) (8).

Antimicrobial susceptibility testing. Antimicrobial Susceptibility Testing (AST) was performed using the VITEK® 2 Compact system with AST-GN93 cards. Minimum inhibitory concentrations (MICs) were obtained for ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, ceftazidime, ceftriaxone, cefepime, aztreonam, ertapenem, meropenem, amikacin, gentamicin, ciprofloxacin, tigecycline, nitrofurantoin, and trimethoprim-sulfamethoxazole. MIC values were interpreted in accordance with the Clinical and Laboratory Standards Institute (CLSI) M100, 34th edition (2024), and classified as susceptible (S), intermediate (I), or Resistant (R) (9).

Phenotypic detection of extended-spectrum β -lactamase (ESBL) production was carried out with CLSI guidelines by assessing MIC patterns generated by the VITEK® 2 Compact system. Isolates showing reduced susceptibility to third-generation cephalosporins and confirmed as ESBL-positive by the automated ESBL detection algorithm embedded in the VITEK® 2 system were classified as ESBL producers, following CLSI criteria and the manufacturer's instructions (9, 10).

Data analysis. Data on respiratory tract infections caused by Gram-negative bacteria were extracted from the WHONET 2022 software and subsequently compiled, tabulated, and processed using Micro-

soft Excel 2021. Descriptive analyses were carried out to characterize the distribution of Gram-negative bacteria responsible for lower respiratory tract infections and to describe their susceptibility patterns to beta-lactam antibiotics during the COVID-19 pandemic. The results were presented as frequencies and percentages in the tables and figure. The distribution of Gram-negative bacteria was analysed at the special level, whereas antimicrobial susceptibility patterns were evaluated according to the categories susceptible, intermediate, and resistant. Comparisons of beta-lactam resistance among major Gram-negative bacteria before and during the COVID-19 pandemic were conducted using Person's chi-square test, with a p-value <0.05 regarded as statistically significant.

RESULTS

Specimen distribution. A total of 569 respiratory specimens meeting the study inclusion criteria were obtained from individuals diagnosed with Gram-negative bacteria-associated lower respiratory tract infections recorded in the WHONET 2022 database between January 2018 and December 2021. Of these, 383 specimens were collected during the pre-pandemic period (2018-2019), whereas 186 specimens were collected in the course of the COVID-19 pandemic (2020-2021).

Sputum specimens represented the most frequently collected sample type, accounting for 464/569 (81.54%), followed by bronchoalveolar lavage specimens (53/569, 9.31%) and bronchial fluid specimens (52/569, 9.14%) (Table 1). The distribution of specimen types was generally comparable between the pre-pandemic and pandemic periods, with sputum remaining the predominant specimen in both intervals (83.03% vs. 78.49%).

Bacterial distribution. Table 2 summarizes the distribution of Gram-negative bacterial isolates recovered from respiratory tract specimens in the period preceding the COVID-19 pandemic (January 2018 – February 2020) and throughout the pandemic era (March 2020 – December 2021).

In total, 383 Gram-negative isolates were detected prior to the pandemic period. *Klebsiella pneumoniae* was the most prevalent species (116/383, 30.29%), followed by *P. aeruginosa* (94/383, 24.54%) and *A. baumannii* (34/383, 8.88%).

Table 1. Distribution of respiratory specimen types before (2018–2019) and during (2020–2021) the COVID-19 pandemic.

Specimen	Year				Before pandemic COVID-19 (%)	During pandemic COVID-19 (%)	Number
	2018	2019	2020	2021			
Sputum	170	148	70	76	83.03%	78.49%	464
Bronchial fluid	12	16	11	13	7.31%	12.90%	52
Bronchoalveolar Lavage	20	17	2	14	9.66%	8.60%	53

Table 2. Distribution of Gram-negative bacteria before and during the COVID-19 pandemic

Bacteria	Number	
	Before pandemic COVID-19	During pandemic COVID-19
<i>Acinetobacter baumannii</i>	34 (8.88%)	51 (27.42%)
<i>Klebsiella pneumoniae</i>	116 (30.29%)	52 (27.96%)
<i>Pseudomonas aeruginosa</i>	94 (24.54%)	47 (25.27%)
<i>Escherichia coli</i>	17 (4.44%)	6 (3.23%)
<i>Aeromonas hydrophilla</i>	3 (0.78%)	-
<i>Aeromonas veronii</i>	1 (0.26%)	-
<i>Achromobacter xyloids</i>	4 (1.04%)	1 (0.54%)
<i>Capnocytophaga spp.</i>	1 (0.26%)	5 (2.69%)
<i>Enterobacter aerogenes</i>	10 (2.61%)	3 (1.61%)
<i>Enterobacter cloacae</i>	28 (7.31%)	4 (2.15%)
<i>Kluyvera cryocrescens</i>	2 (0.52%)	-
<i>Kluyvera oxytoca</i>	5 (1.31%)	-
<i>Pantoea spp</i>	2 (0.52%)	-
<i>Burkholderia cepacia</i>	29 (7.57%)	1 (0.54%)
<i>Burkholderia pseudomallei</i>	2 (0.52%)	-
<i>Ralstonia mannitolilytica</i>	1 (0.26%)	-
<i>Sphingomonas paucimobilis</i>	10 (2.61%)	4 (2.15%)
<i>Bordetella bronchiseptica</i>	1 (0.26%)	-
<i>Pseudomonas luteola</i>	2 (0.52%)	-
<i>Chryseobacterium indologenes</i>	1 (0.26%)	1 (0.54%)
<i>Elizabethkingia meningoseptica</i>	-	1 (0.54%)
<i>Proteus mirabilis</i>	4 (1.04%)	1 (0.54%)
<i>Providencia rettgeri</i>	2 (0.52%)	-
<i>Providencia stuartii</i>	1 (0.26%)	1 (0.54%)
<i>Citrobacter freundii</i>	-	1 (0.54%)
<i>Pantoea agglomerans</i>	2 (0.52%)	-
<i>Pluralibacter gergoviae</i>	1 (0.26%)	2 (1.08%)
<i>Raoultella ornithinolytica</i>	-	1 (0.54%)
<i>Klebsiella ozanae</i>	4 (1.04%)	-
<i>Serratia marcescens</i>	3 (0.78%)	3 (1.61%)
<i>Pseudomonas fluorescens</i>	1 (0.26%)	-
<i>Pseudomonas putida</i>	2 (0.52%)	1 (0.54%)
Number	383	186

During the pandemic, 186 Gram-negative isolates were recovered. *K. pneumoniae* remained the most frequently isolated organism (52/186, 27.96%), followed by *A. baumannii* (51/186, 27.42%) and *P. aeruginosa* (47/186, 25.27%). Relative to the pre-pandemic interval, the share of *A. baumannii* rose substantially, whereas the proportions of several other Gram-negative species declined.

Overall, 29 Gram-negative bacterial species were identified before the pandemic, whereas only 19 species were recovered during the pandemic, indicating a reduction in bacterial diversity among respiratory isolates. The distribution of Gram-negative bacterial species across the two study periods is depicted in Figs. 1 and 2.

The overall distribution of Gram-negative bacterial species differed significantly when comparing the pre-pandemic and pandemic periods, as determined by Pearson's Chi-square test ($\chi^2 = 24.70$, $df = 3$, $p < 0.001$).

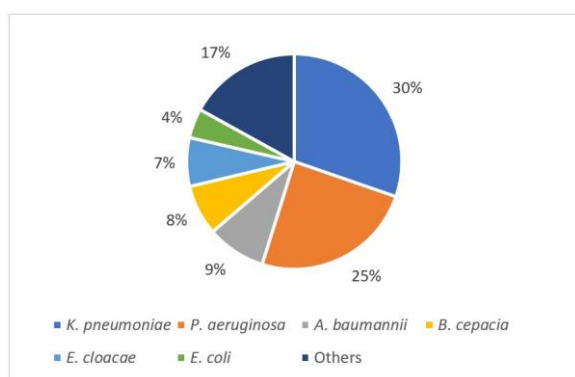


Fig. 1. Percentage distribution of Gram-negative bacteria before the COVID-19 pandemic

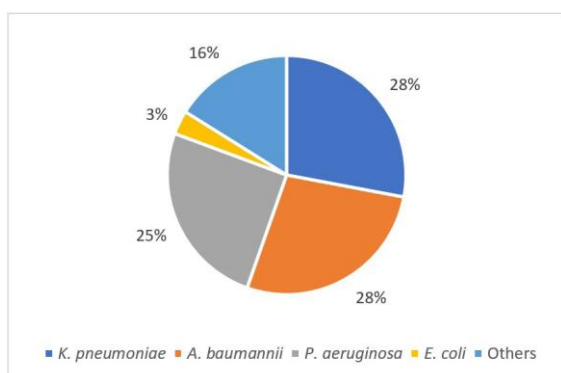


Fig. 2. Percentage distribution of Gram-negative Bacteria during the COVID-19 Pandemic

Bacterial resistance to beta-lactam antibiotics in the pre-Covid-19 era. Table 3 summarizes beta-lactam resistance profiles among Gram-negative bacterial isolates to five antibiotics, namely ampicillin-sulbactam, cefepime, ceftazidime, meropenem, and piperacillin-tazobactam, during the pre-pandemic period (January 2018 – February 2020).

In total, 383 Gram-negative isolates were included in the analysis. The predominant species were *K. pneumoniae* ($n = 116$), *P. aeruginosa* ($n = 94$), *A. baumannii* ($n = 34$), *Burkholderia cepacia* ($n = 29$), *Enterobacter cloacae* ($n = 28$), and *Escherichia coli* ($n = 17$).

Among the predominant bacterial species, resistance rates varied considerably across the tested beta-lactam antibiotics.

No meropenem-resistant isolate was identified among *E. coli* (0/17, 0% resistance).

The highest meropenem resistance was observed in *A. baumannii*, with 24 of 34 isolates resistant (70.58%).

High resistance was also observed in *Burkholderia cepacia* to piperacillin-tazobactam (24/29, 82.75%) and in *P. aeruginosa* to piperacillin-tazobactam (37/92, 40.21%) and meropenem (34/94, 36.17%). In contrast, *Enterobacter cloacae* demonstrated relatively low resistance rates to all tested beta-lactam antibiotics, ranging from 7.14% to 14.28%.

Resistance rates among less frequently isolated bacterial species need to be viewed cautiously, given the small number of isolates available.

Bacterial resistance to beta-lactam antibiotics during the COVID-19 pandemic. Based on Table 4, data are presented on beta-lactam resistance profiles in Gram-negative bacteria to agents such as ampicillin-sulbactam, cefepime, ceftazidime meropenem, and piperacillin-tazobactam during the COVID-19 pandemic (2020-2021). In total, 186 isolates were identified, with the four most common species being *Acinetobacter baumannii*, *K. pneumoniae*, *P. aeruginosa*, and *E. coli*. These isolates underwent susceptibility testing against beta-lactam antibiotics including ampicillin-sulbactam, cefepime, ceftazidime, meropenem, and piperacillin-tazobactam. The data reveal heterogeneous resistance rates and a modest increase in resistance for some species to the tested antibiotics relative to the pre-COVID-19 period. Meropenem remained 100% effective against *E. coli* both before and throughout the COVID-19 pandemic.

Table 3. Bacterial resistance to beta-lactam antibiotics before the COVID-19 pandemic

Bacteria	Resistance				
	Ampicillin-Sulbactam	Cefepime	Ceftazidime	Meropenem	Piperacillin-Tazobactam
<i>Acinetobacter baumannii</i> (n = 34)	16/28 (57.14%)	18/28 (64.28%)	22/34 (64.70%)	24/34 (70.58%)	22/34 (64.70%)
<i>Klebsiella pneumoniae</i> (n = 116)	38/106 (35.84%)	16/104 (15.38%)	43/115 (37.39%)	14/116 (12.06%)	25/116 (21.55%)
<i>Pseudomonas aeruginosa</i> (n = 94)	-	12/78 (15.38%)	21/93 (22.58%)	34/94 (36.17%)	37/92 (40.21%)
<i>Escherichia coli</i> (n = 17)	7/17 (41.17%)	5/16 (31.25%)	9/17 (52.94%)	0/17 (0%)	3/17 (17.64%)
<i>Aeromonas hydrophilla</i> (n = 3)	3/3 (100%)	0/3 (0%)	0/3 (0%)	-	0/3 (0%)
<i>Aeromonas veronii</i> (n = 1)	-	-	-	-	-
<i>Achromobacter xyloids</i> (n = 4)	-	2/4 (50%)	0/4 (0%)	0/4 (0%)	2/4 (50%)
<i>Capnocytophaga</i> spp. (n = 1)	-	-	-	-	-
<i>Enterobacter aerogenes</i> (n = 10)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)	0/10 (0%)
<i>Enterobacter cloacae</i> (n = 28)	4/28 (14.28%)	2/28 (7.14%)	3/28 (10.71%)	2/28 (7.14%)	3/28 (10.71%)
<i>Kluyvera cryocrescens</i> (n = 2)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
<i>Kluyvera oxytoca</i> (n = 5)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)	0/5 (0%)
<i>Pantoea</i> spp (n = 2)	-	0/1 (0%)	0/1 (0%)	0/1 (0%)	-
<i>Burkholderia cepacia</i> (n = 29)	-	4/28 (14.28%)	3/28 (10.71%)	8/28 (28.56%)	24/29 (82.75%)
<i>Burkholderia pseudomallei</i> (n = 2)	-	-	1/1 (100%)	1/1 (100%)	-
<i>Ralstonia mannitolilytica</i> (n = 1)	-	-	-	-	-
<i>Sphingomonas paucimobilis</i> (n = 10)	-	0/4 (0%)	0/6 (0%)	3/7 (42.85%)	1/4 (25%)
<i>Bordetella bronchiseptica</i> (n = 1)	-	0/1 (0%)	0/1 (0%)	0/1 (0%)	-
<i>Pseudomonas luteola</i> (n = 2)	-	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
<i>Chryseobacterium indologenes</i> (n = 1)	-	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
<i>Proteus mirabilis</i> (n = 4)	0/4 (0%)	0/3 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
<i>Providencia rettgeri</i> (n = 2)	1/2 (50%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
<i>Providencia staurtii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Pantoea agglomerans</i> (n = 2)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
<i>Pluralibacter gergoviae</i> (n = 1)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Klebsiella ozanae</i> (n = 4)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
<i>Serratia marcescens</i> (n = 3)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
<i>Pseudomonas fluorescens</i> (n = 1)	-	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Pseudomonas putida</i> (n = 2)	-	0/2 (0%)	0/2 (0%)	2/2 (100%)	2/2 (100%)

Noted: n: number of resistant isolates

Resistance percentages were calculated based on the number of isolates tested for each antibiotic.

A. baumannii resistance to piperacillin-tazobactam rose to 92.15% compared with the pre-pandemic period. For other bacteria, estimates also vary, but the data are limited.

To compare resistance patterns across the pre-pandemic and pandemic periods, resistance rates of the three predominant Gram-negative pathogens were evaluated using Pearson's Chi-square test (Table 5).

Among *A. baumannii*, resistance to cefepime ($p = 0.036$), ceftazidime ($p = 0.018$), and piperacillin-ta-

zobactam ($p = 0.002$) increased significantly during the COVID-19 pandemic. Although meropenem resistance also increased (70.6% vs. 86.3%), this difference did not achieve statistical significance ($p = 0.067$). By contrast, no significant changes in beta-lactam resistance were observed for *K. pneumoniae* (all $p > 0.05$). For *P. aeruginosa*, resistance to piperacillin-tazobactam declined significantly during the pandemic (40.2% vs. 22.7%, $p = 0.041$), whereas resistance to cefepime, ceftazidime, and meropenem remained unchanged (all $p > 0.05$).

Table 4. Bacterial resistance to beta-lactam antibiotics during the COVID-19 pandemic

Bacteria	Resistance				
	Ampicillin-Sulbactam	Cefepime	Ceftazidime	Meropenem	Piperacillin - Tazobactam
<i>Acinetobacter baumannii</i> (n = 51)	23/51 (45.09%)	43/51 (84.31%)	44/51 (86.27%)	44/51 (86.27%)	47/51 (92.15%)
<i>Klebsiella pneumoniae</i> (n = 52)	18/51 (35.29%)	10/52 (19.23%)	21/51 (41.17%)	7/52 (13.46%)	12/51 (23.52%)
<i>Pseudomonas aeruginosa</i> (n = 47)	-	9/47 (19.14%)	9/44 (20.45%)	12/47 (25.53%)	10/44 (22.72%)
<i>Escherichia coli</i> (n = 6)	3/6 (50%)	1/6 (16.67%)	2/6 (33.33%)	0/6 (0%)	2/6 (33.33%)
<i>Achromobacter xyloids</i> (n = 1)	-	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Capnocytophaga</i> spp. (n = 5)	-	-	-	-	-
<i>Enterobacter aerogenes</i> (n = 3)	1/3 (33.33%)	0/3 (0%)	1/3 (33.33%)	0/3 (0%)	2/3 (66.67%)
<i>Enterobacter cloacae</i> (n = 4)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)	0/4 (0%)
<i>Burkholderia cepacia</i> (n = 1)	-	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
<i>Sphingomonas paucimobilis</i> (n = 4)	-	-	0/2 (0%)	2/2 (100%)	0/1 (0%)
<i>Chryseobacterium indologenes</i> (n = 1)	-	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
<i>Elizabethkingia meningoseptica</i> (n = 1)	-	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
<i>Proteus mirabilis</i> (n = 1)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Providencia stuartii</i> (n = 1)	1/1 (100%)	0/1 (0%)	0/1 (0%)	0/1 (0%)	0/1 (0%)
<i>Citrobacter freundii</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	0/1 (0%)	1/1 (100%)
<i>Pluralibacter gergoviae</i> (n = 2)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)	0/2 (0%)
<i>Raoultella ornithinolytica</i> (n = 1)	1/1 (100%)	0/1 (0%)	1/1 (100%)	1/1 (100%)	1/1 (100%)
<i>Serratia marcescens</i> (n = 3)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)	0/3 (0%)
<i>Pseudomonas putida</i> (n = 1)	-	1/1 (100%)	1/1 (100%)	1/1 (100%)	1/1 (100%)

Noted: n: number of resistant isolates

Resistance percentages were derived by dividing the number of resistant isolates by the total number of isolates tasted for each antibiotic and expressing this value as percentage.

Table 5. Comparison of beta-lactam resistance among major Gram-negative bacteria before and during the COVID-19 pandemic

Bacteria	Antibiotic	Before pandemic	During pandemic	χ^2	p-value
		n/N (%)	n/N (%)		
<i>Acinetobacter baumannii</i>	Ampicillin-sulbactam	16/28 (57.1)	23/51 (45.1)	0.99	0.320
	Cefepime	18/28 (64.3)	43/51 (84.3)	4.39	0.036
	Ceftazidime	22/34 (64.7)	44/51 (86.3)	5.61	0.018
	Meropenem	24/34 (70.6)	44/51 (86.3)	3.36	0.067
	Piperacillin-tazobactam	22/34 (64.7)	47/51 (92.2)	9.68	0.002
<i>Klebsiella pneumoniae</i>	Ampicillin-sulbactam	38/106 (35.8)	18/51 (35.3)	0.00	0.950
	Cefepime	16/104 (15.4)	10/52 (19.2)	0.35	0.550
	Ceftazidime	43/115 (37.4)	21/51 (41.2)	0.22	0.640
	Meropenem	14/116 (12.1)	7/52 (13.5)	0.06	0.810
	Piperacillin-tazobactam	25/116 (21.6)	12/51 (23.5)	0.07	0.790
<i>Pseudomonas aeruginosa</i>	Cefepime	12/78 (15.4)	9/47 (19.1)	0.31	0.580
	Ceftazidime	21/93 (22.6)	9/44 (20.5)	0.08	0.780
	Meropenem	34/94 (36.2)	12/47 (25.5)	1.65	0.200
	Piperacillin-tazobactam	37/92 (40.2)	10/44 (22.7)	4.18	0.041

Noted: Data are presented as number of resistant isolates/total tested isolates (n/N) with percentage in parentheses. Statistical analysis was performed using Pearson's Chi-square test. A p-value < 0.05 was considered statistically significant.

DISCUSSION

Specimen distribution. A marked disparity in specimen counts was observed among sputum, bronchial fluid, and bronchoalveolar lavage samples. Overall comparison showed that although specimen proportions varied across types, the difference between pre-pandemic and pandemic periods was not statistically emphasized in this study.

However, there was a decrease in specimens in the COVID-19 period relative to the pre-pandemic era. This is because during the pandemic, the health sector focused more on the COVID-19 phenomenon than on lower respiratory tract infections attributable to Gram-negative bacteria. Furthermore, more sputum specimens were examined. This is because sputum specimens originate from the lower respiratory tract and are therefore considered representative of respiratory tract infections, particularly Gram-negative bacteria. Furthermore, the non-invasive procedure and more affordable cost are also reasons for the higher number of samples using sputum specimens (11).

Bacterial distribution. Before the COVID-19 pandemic (2018-2019), 383 isolates of Gram-negative bacteria were identified, whereas during the pandemic period (2020-2021) the isolate count declined to 186. Furthermore, the number of identified Gram-negative bacterial species also decreased, from 29 species before the pandemic to 19 during the pandemic. The decrease in the number of isolates and the diversity of bacterial species is thought to be related to the widespread implementation of health protocols over the course of the COVID-19 pandemic. Various preventative interventions, including mask use, social distancing, restrictions on mobility and social interaction, and increased hygiene practices, contributed to reducing the transmission of respiratory infections. Although these protocols were initially intended to reduce the spread of COVID-19, their implementation also indirectly reduced the occurrence of other respiratory infections, including those caused by Gram-negative bacteria (12, 13). During the pre-COVID-19 period (2018-2019), 383 Gram-negative bacterial isolates were documented. The three leading species were *K. pneumoniae* (30.29%), *P. aeruginosa* (24.54%), and *A. baumannii* (8.88%) (14).

These results are consistent with the work of Santella et al. (2021), who documented 889 cases in 2018

and 930 cases in 2019. In this study, *A. baumannii* was the most frequently found bacterium, accounting for 20.1% in 2018 and 17.7% in 2019. Meanwhile, *K. pneumoniae* increased from 8.1% in 2018 to 11.7% in 2019. In general, the distribution pattern of Gram-negative bacteria responsible for lower respiratory tract infections in this study showed a similar trend to previous studies during the pre-COVID-19 period (15).

In the COVID-19 period (2020-2021), 186 infection episodes were documented, with *K. pneumoniae* emerging as the leading pathogen (27.96%), followed by *A. baumannii* (27.32%), *P. aeruginosa* (25.27%), and *E. coli* (3.23%).

The distribution pattern of Gram-negative bacteria in this study showed a similar trend to that reported in the investigation by Khadak et al. conducted in Nepal (2022). That study reported 112 cases of respiratory tract infections, with the most common pathogen being *K. pneumoniae* (37.93%), followed by *A. baumannii* (29.31%), *E. coli* (11.21%), and *P. aeruginosa* (9.48%). Thus, *K. pneumoniae*, *A. baumannii*, and *P. aeruginosa* remain the leading Gram-negative bacteria causing lower respiratory tract infections during the COVID-19 period (16, 17).

Although the overall number of cases caused by Gram-negative bacteria decreased over the course of the COVID-19 pandemic, infections remained at a high rate. This decrease is thought to be related to a shift in healthcare priorities during the pandemic, with the majority of resources and clinical attention focused on COVID-19 prevention, vaccination, and management.

During the pandemic, *K. pneumoniae* remained the most common Gram-negative pathogen implicated in lower respiratory tract infections. The high prevalence of this bacterium may be attributed to its frequent presence in the hospital settings, increasing the risk of exposure to patients, particularly inpatients and those with compromised immune systems.

Furthermore, *K. pneumoniae* possesses various virulence determinants that enhance its capacity to establish infection and withstand antibiotic therapy. This bacterium is capable of producing beta-lactamase enzymes, which play a role in increasing resistance to beta-lactam antibiotics. *K. pneumoniae* also possesses a polysaccharide capsule that helps evade the host immune response, as well as a fimbrial structure that facilitates bacterial adhesion to host cells. The lipopolysaccharide structure of the bacterial outer mem-

brane also plays a role in inducing an inflammatory response. Furthermore, the ability to secrete siderophores allows bacteria to bind and obtain iron from the host, thus supporting their growth and survival (18, 19).

Bacterial resistance. In this study, *A. baumannii* showed varying levels of resistance to the five antibiotics before the COVID-19 pandemic, ranging from 57.14% to ampicillin-sulbactam decreased to 45.09%, while resistance to other antibiotics increased, particularly to piperacillin-tazobactam, which reached 92.15%.

Statistical analysis confirmed that several of these changes were significant, particularly for cefepime, ceftazidime, and piperacillin-tazobactam ($p < 0.05$), supporting a genuine alteration in resistance profiles rather than random fluctuation.

These findings align with a study in Slovakia that reported that before the COVID-19 pandemic, *A. baumannii* resistance to ampicillin-sulbactam reached nearly 90%. Furthermore, resistance to ceftazidime, cefepime, and piperacillin-tazobactam was reported to approach 95%. In the COVID-19 era, however, resistance patterns have shifted, characterized by a decreased in resistance to ampicillin-sulbactam to below 50%, while resistance to ceftazidime, cefepime, and piperacillin-tazobactam has reached 100% (20).

Another study in Romania also showed an increase in *A. baumannii* resistance to meropenem. In 2018 and 2019, resistance rates reached 75% and 72.22%, respectively, before increasing to 93.75% in 2020 and 97.96% in 2021 (21).

The resistance pattern of *K. pneumoniae* differed from *A. baumannii*. Before the COVID-19 pandemic, resistance rates were relatively lower, including 12.06% for meropenem and 37.39% for ceftazidime. During the pandemic, only slight increases were observed (meropenem 13.46%, ceftazidime 41.17%).

However, statistical testing showed no significant differences across all antibiotics ($p > 0.05$), indicating that observed variations may reflect natural fluctuation rather than true epidemiological change.

This contrasts with findings from Egypt reporting higher baseline resistance (49% for ceftazidime and cefepime) (22). A study in Slovakia also identified carbapenemase-producing strains (KPC and NDM) with very high resistance rates, particularly affecting β -lactam antibiotics (23).

For *P. aeruginosa*, resistance decreased for most antibiotics during the pandemic, except for ceftazi-

dime. Notably, piperacillin-tazobactam resistance significantly decreased during the pandemic ($p = 0.041$).

This differs from a study in China reporting relatively stable resistance trends across pre- and intra-pandemic periods (24).

Regarding *E. coli*, resistance before the pandemic ranged from 17.64% to 52.94%, while meropenem remained fully active (100% susceptibility) in both periods. During the pandemic, resistance varied, with the highest value at 50% for ampicillin-sulbactam. A study in Romania reported lower carbapenem resistance (5.26%) (25), whereas an Egyptian study reported substantially higher resistance across all antibiotics during the pandemic period.

Overall, variability in bacterial resistance across studies may be influenced by differences in antibiotic usage policies, healthcare infrastructure, antimicrobial stewardship programs, socioeconomic conditions, and environmental factors such as climate and infection control practices (26).

This study is subject to several constraints. First, the lack of patient demographic information restricts our capacity to explore potential differences in bacterial distribution and antimicrobial resistance across various patient subgroups. These variables are known to influence infection risk and resistance patterns, particularly in elderly populations or immunocompromised patients.

Second, there is a lack of comprehensive clinical data, encompassing comorbid conditions, disease severity, and COVID-19 status. This hinders the interpretations of whether the identified bacterial isolates represent colonization or actual infection. Consequently, the clinical relevance of some of these isolates cannot be definitively determined.

Third, information regarding patient care settings, such as intensive care units (ICUs), inpatient wards, or outpatient clinics, is unavailable. This constraint reduces our capacity to evaluate the influence of different healthcare environments on antimicrobial resistance profiles, given that ICU settings are often associated with higher prevalence rates of multi-drug-resistant organism.

Finally, because this was a retrospective investigation conducted at a single center, the result may not be fully applicable to other institutions or geographic areas. Despite these constraints, the study offers important surveillance evidence on the distribution of Gram-negative bacteria and their resistance trajectories across the pre-pandemic and COVID-19 periods.

CONCLUSION

This study shows that *K. pneumonia*, *P. aeruginosa*, and *A. baumannii*, consistently emerged as the predominant Gram-negative pathogens responsible for lower respiratory tract infections in both the pre-COVID-19 and during COVID-19 pandemic. At the same time, the overall number of isolates decreased during the pandemic.

Most Gram-negative isolates in this study showed resistance rates below 50% to the evaluated beta-lactam antibiotics. Nonetheless, *A. baumannii* demonstrated a statistically significant rise in resistance to ceftazidime, cefepime, and piperacillin-tazobactam during the COVID-19 pandemic.

In general, the distributions of bacterial species and their antimicrobial resistance profiles was broadly comparable between the two periods. The main exception was *A. baumannii*, which showed a marked rise in both prevalence and resistance.

ACKNOWLEDGEMENTS

The authors express their gratitude to the Department of Microbiology, Faculty of Medicine, Universitas Indonesia, for providing the isolate data used in this study. All authors have approved the final version of this manuscript and declare that they have no conflicts of interest related to this research.

REFERENCES

- Harapan BN, Harapan T, Theodora L, Anantama NA. From archipelago to pandemic battleground: unveiling Indonesia's COVID-19 crisis. *J Epidemiol Glob Health* 2023; 13: 591-603.
- Shi Y, Wang G, Cai XP, Deng JW, Zheng L, Zhu HH, et al. An overview of COVID-19. *J Zhejiang Univ Sci B* 2020; 21: 343-360.
- Fazel P, Sedighian H, Behzadi E, Kachuei R, Imani Fooladi AA. Interaction between SARS-CoV-2 and pathogenic bacteria. *Curr Microbiol* 2023; 80: 223.
- Wu G, Lu J, Liu D, He Y. Characteristics and risk factors of secondary bacterial infections in COVID-19 patients. *Antimicrob Steward Healthc Epidemiol* 2023; 3(1): e156.
- Garcia-Bustos V, Cabañero-Navalón MD, Salavert Lletí M. Resistance to beta-lactams in Gram-negative bacilli: relevance and potential therapeutic alternatives. *Rev Esp Quimioter* 2022; 35(Suppl 2): 1-15.
- Wulandari RA, Fauzia S, Kurniasari F. Investigations on the risk factors of Acute Respiratory Infections (ARIs) among under-five children in Depok City, Indonesia. *Ann Ig* 2024; 36: 15-25.
- Sannathimmappa MB, Nambiar V, Aravindakshan R, Al-Kasaby NM. Profile and antibiotic-resistance pattern of bacteria isolated from endotracheal secretions of mechanically ventilated patients at a tertiary care hospital. *J Educ Health Promot* 2021; 10: 195.
- Cleveland Clinic. Upper respiratory infection [Internet]. Cleveland (OH): Cleveland Clinic; 2021 [cited 2024 Oct 9]. Available from: <https://my.clevelandclinic.org/health/articles/4022-upper-respiratory-infection>
- Oliveira J. Gram-negative bacteria [Internet]. U.S. National Library of Medicine; 2023 [cited 2025 Mar 11]. Available from: <https://www.ncbi.nlm.nih.gov/books/NBK538213/>.
- Duan N, Du J, Huang C, Li H. Microbial distribution and antibiotic susceptibility of lower respiratory tract infections patients from pediatric ward, adult respiratory ward, and respiratory intensive care unit. *Front Microbiol* 2020; 11: 1480.
- Centers for Disease Control and Prevention. About *Pseudomonas aeruginosa* disease [Internet]. Atlanta (GA): Centers for Disease Control and Prevention; 2024 [cited 2024 Oct 9]. Available from: <https://www.cdc.gov/pseudomonas-aeruginosa/about/index.html>
- Blakeborough L, Watson JS. The importance of obtaining a sputum sample and how it can aid diagnosis and treatment. *Br J Nurs* 2019; 28: 295-298.
- Altamimi I, Binkhamis K, Alhumimidi A, Alabdulkarim IM, Almugren A, Alhems H, et al. Decline in ESBL Production and Carbapenem Resistance in Urinary Tract Infections among Key Bacterial Species during the COVID-19 Pandemic. *Antibiotics (Basel)* 2024; 13: 216.
- Tanislav C, Kostev K. Fewer non-COVID-19 respiratory tract infections and gastrointestinal infections during the COVID-19 pandemic. *J Med Virol* 2021; 94: 298-302.
- Santella B, Serretiello E, De Filippis A, Veronica F, Iervolino D, Dell'Annunziata F, et al. Lower Respiratory Tract Pathogens and Their Antimicrobial Susceptibility Pattern: A 5-Year Study. *Antibiotics (Basel)* 2021; 10: 851.
- Maebed AZM, Gaber Y, Bakeer W, Dishisha T. Microbial etiologies of ventilator-associated pneumonia (VAP) in intensive care unit of Beni-Suef University's Hospital. *Beni Suef Univ J Basic Appl Sci* 2021; 10: 41.
- Khadka S, Barakoti A, Adhikari RP, Khanal LK, Sap-

- kota J. Positive Bacterial Culture among Lower Respiratory Tract Specimens of Patients in a Tertiary Care Center: A Descriptive Cross-sectional Study. *JNMA J Nepal Med Assoc* 2022; 60: 384-388.
18. Kumar A, Chakravorty S, Yang T, Russo TA, Newton SM, Klebba PE. Siderophore-mediated iron acquisition by *Klebsiella pneumoniae*. *J Bacteriol* 2024; 206(5): e0002424.
 19. Jalali Y, Liptáková A, Jalali M, Payer J. Moving toward Extensively Drug-Resistant: Four-Year Antimicrobial Resistance Trends of *Acinetobacter baumannii* from the Largest Department of Internal Medicine in Slovakia. *Antibiotics (Basel)* 2023; 12: 1200.
 20. Borcan AM, Rotaru E, Caravia LG, Filipescu MC, Simoiu M. Trends in Antimicrobial Resistance of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* from Bloodstream Infections: An Eight-Year Study in a Romanian Tertiary Hospital. *Pharmaceuticals (Basel)* 2025; 18: 948.
 21. Abdelaziz Abdolmoneim S, Mohamed Ghazy R, Anwar Sultan E, Hassaan MA, Anwar Mahgoub M. Antimicrobial resistance burden pre- and post-COVID-19 pandemic with mapping the multidrug resistance in Egypt: a comparative cross-sectional study. *Sci Rep* 2024; 14: 7176.
 22. Ficik J, Andrezál M, Drahovská H, Böhmer M, Szemes T, Liptáková A, et al. Carbapenem-Resistant *Klebsiella pneumoniae* in COVID-19 Era-Challenges and Solutions. *Antibiotics (Basel)* 2023; 12: 1285.
 23. Xia J, Lu L, Zhao KL, Zeng QL. Resistance Transition of *Pseudomonas aeruginosa* in SARS-CoV-2-Uninfected Hospitalized Patients in the Pandemic. *Infect Drug Resist* 2023; 16: 6717-6724.
 24. Golli AL, Popa SG, Ghenea AE, Turcu FL. The Impact of the COVID-19 Pandemic on the Antibiotic Resistance of Gram-Negative Pathogens Causing Bloodstream Infections in an Intensive Care Unit. *Biomedicines* 2025; 13: 379.
 25. Rahbe E, Watier L, Guillemot D, Glaser P, Opatowski L. Determinants of worldwide antibiotic resistance dynamics across drug-bacterium pairs: a multivariable spatial-temporal analysis using ATLAS. *Lancet Planet Health* 2023; 7(7): e547-e557.
 26. Lai CC, Chen SY, Ko WC, Hsueh PR. Increased antimicrobial resistance during the COVID-19 pandemic: a systematic review and meta-analysis of hospital-acquired infections. *Int J Antimicrob Agents* 2021; 57: 106324.