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Distribution of ESBL-producing and non-ESBL-producing *Klebsiella pneumoniae* isolated from sputum specimens in the Zainoel Abidin General Hospital, Banda Aceh, Indonesia

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Abstract. *Suhartono S, Hayati Z, Mahdani W, Andini F. 2024. Distribution of ESBL-Producing and Non-ESBL-Producing* Klebsiella pneumoniae *isolated from sputum specimens in the Zainoel Abidin General Hospital, Banda Aceh, Indonesia. Biodiversitas 25: 3247-3254. Klebsiella pneumoniae,* a significant nosocomial pathogen, particularly in the case of strains producing extended-spectrum beta-lactamase (ESBL), was the focus of this study. The study aimed to determine the distributions stratified by patients' age, sex, hospital wards, and the antimicrobial susceptibility pattern of ESBL and non-ESBL *K. pneumoniae* isolated from expectorate sputum at Zainoel Abidin General Hospital (RSUD dr. Zainoel Abidin), Banda Aceh, Indonesia, from January 2021 to January 2024. The prevalence of ESBL-producing *K. pneumoniae* was 79.84% (103/129 isolates), with the highest incidence found in patients aged >45 years (74.76%) and males (67.96%). The prevalence of non-ESBL-producing *K. pneumoniae* was 20.16% (26 isolates), with the highest incidence in patients aged >45 years (76.92%) and males (62.23%). The surgical ward had the highest incidence of ESBL-producing K. pneumoniae (14.56%), followed by the cardiopulmonary ward (14.56%). The cardiopulmonary ward also had the highest incidence of non-ESBL-producing *K. pneumoniae* (38.47%). The ESBL-producing *K. pneumoniae* isolates were more sensitive to amikacin than non-ESBL isolates, while the non-ESBL-producing *K. pneumoniae* isolates were sensitive to cefotaxime, amikacin, and tobramycin. The increasing prevalence of ESBL-producing K. pneumoniae underscores the significance of the study's findings, providing valuable insights into the epidemiology and antibiotic susceptibility profiles of *K. pneumoniae* in this clinical setting and the importance of reasonable antibiotic use.

Keywords: Antibiotic susceptibility, ESBL, *Klebsiella pneumoniae*, nosocomial infection, sputum

Abbreviations: ESBL: Extended-Spectrum Beta-Lactamase

INTRODUCTION

Klebsiella pneumoniae, a gram-negative bacterium of the Enterobacteriaceae family, is a major nosocomial pathogen, particularly concerning due to its association with extended-spectrum beta-lactamase (ESBL) productions, which confers resistance to many antibiotics. The bacterial pathogen is characterized by its opportunistic nature and capacity to inhabit different surfaces and medical equipment within hospitals, thus playing a significant role in infections acquired in healthcare settings. The emergence and spread of *K. pneumoniae* strains producing ESBL have become a major global health challenge over the past two decades. The emergence of such strains complicates treatment plans since they are resistant to antimicrobial drugs. ESBL-producing K pneumoniae is associated with higher morbidity, mortality, and healthcare costs, posing a global challenge to healthcare systems. Patients with ESBL-producing *K. pneumoniae* infections necessitate using more costly antibiotics due to the resistance of these strains to standard treatments, leading to prolonged hospitalization and elevating the risk of complications. This global challenge underscores the urgency and importance of our research in understanding and combating this issue.

In recent years, the emergence of ESBL-producing *K. pneumoniae* has become a significant concern in healthcare settings worldwide. ESBLs are enzymes that confer resistance to a broad range of beta-lactam antibiotics, including penicillins, cephalosporins, and monobactams (Jiang et al. 2020). Genes responsible for producing extended-spectrum beta-lactamases (ESBLs), such as *bla*CTX-M, *bla*TEM, and *bla*SHV (Shrief et al. 2022), are commonly located on mobile genetic elements, facilitating their transfer between other bacterial populations (Jones et al. 2021). Carbapenems are commonly considered the last resort for treating infections caused by ESBL-producing *K. pneumoniae*. Nevertheless, the therapeutic use of this drug has been hampered by the emergence of carbapenem-resistant bacteria (Ding et al. 2019); the need for a robust antibiotic stewardship program and strong infection control measures has been highlighted by the findings that carbapenem-resistant *K. pneumoniae* is increasingly found in many hospitals across Indonesia (Muztika et al. 2020; Hayati et al. 2021).

The global prevalence of ESBL-producing *K. pneumoniae* varies, with reported rates ranging from 20 to 60% (Sinanjung et al. 2020). Furthermore, the association

between ESBL production and severe infections has been well-documented, with patients infected by ESBL-producing bacteria experiencing a more severe disease course compared to those infected with non-ESBL isolates (Devipalanisamy et al. 2021; Zaniani et al. 2022). The significant variation in this range can be ascribed to disparities in the utilization of antibiotics, implementation of infection control measures, and monitoring techniques in different healthcare settings and geographic areas (Manuaba et al. 2021). Several epidemiological studies in Indonesia have reported the presence of ESBL-producing *K. pneumoniae* in various clinical samples, including sputum (Hayati et al. 2019; Manuaba et al. 2021). However, there is insufficient epidemiological data on the prevalence and antibiotic resistance profiles of ESBL-producing and non-ESBL-producing *K pneumoniae* in sputum samples from patients at Zainoel Abidin General Hospital, the largest public hospital in Aceh Province, is a tertiary health and education referral facility (Hayati et al. 2019; Suhartono et al. 2021). By comparing the prevalence and susceptibility patterns of ESBL and non-ESBL-producing *K. pneumoniae*, healthcare facilities can develop targeted interventions to reduce the spread of resistant organisms. This requires detecting changes in resistance patterns of ESBL and non-ESBL-producing *K. pneumoniae* over time. Additionally, knowledge of the antimicrobial susceptibility patterns of ESBL and non-ESBL-producing *K. pneumoniae* is essential for accurate and timely susceptibility testing to guide the selection of appropriate empirical therapy as clinicians can optimize treatment outcomes and minimize the use of broad-spectrum antibiotics.

This study assessed the distribution of ESBL-producing and non-ESBL-producing strains of *K. pneumoniae* over three years. The analysis focused on stratifying the distributions according to the characteristics of the patients (age, sex, and hospital wards) and the susceptibility pattern to antibiotics. This study provides valuable insights into the involvement of *K. pneumoniae* in respiratory tract infections, a common type of nosocomial infection.

MATERIALS AND METHODS

Study design and setting

The ESBL and non-ESBL-producing *Klebsiella pneumoniae* data was collected from sputum specimens at the Clinical Microbiology Laboratory of Zainoel Abidin General Hospital (RSUD dr. Zainoel Abidin), Banda Aceh, Indonesia, from January 2021 to January 2024, using a laboratory observation procedure. The Health Research Ethics Committee of Zainoel Abidin General Hospital approved this study (No. 258/ETIK-RSUDZA/2023).

Sample collection, bacterial isolation, observation, and identification

Specimens of patients' sputum were obtained and processed routinely using standard microbiological methods. The specimens were cultured on blood agar (BA), MacConkey agar (MCA), and chocolate agar (CA) plates following the manufacturer's instructions. Subsequently, they were incubated at 37°C for 24 hours before pure isolated colonies on the BA and MCA were gram-stained and examined under a light microscope using 1000x magnification.

Additional identification and antibiotic susceptibility were conducted using VITEK® 2 Compact (Biomeriux, Lyon, France). A pure bacterial colony isolated from clinical samples was suspended in NaCl 0.45 percent solution, which was equivalent to 1.8-2.2 McFarland Standard solution, and then inoculated into appropriate cassettes of GN (Gram-negative) and AST (antimicrobial susceptibility testing), respectively, for identification and antimicrobial susceptibility testing.

Antibiotic sensitivity testing

Antibiotic sensitivity was tested against a panel of antibiotics, including amoxicillin, ampicillin, amoxicillin/clavulanic, piperacillin/tazobactam, cefoxitin, cefotaxime, ceftazidime, ceftriaxone, cefoperazone/sulbactam, doripenem, imipenem, meropenem, amikacin, gentamicin, tobramycin, levofloxacin, doxycycline. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines.

Data analysis

The distribution of ESBL-producing and non-ESBL-producing *K. pneumoniae* isolates was descriptively analyzed based on patients' age, sex, and hospital ward. All data were tabulated using Microsoft Excel to generate descriptive information in tables or charts. Statistical analysis was performed using the chi-square test or Fisher''s exact test when appropriate. All tests performed using XLStat 2023 (Addinsoft, New York, USA) were considered statistically significant at a $p \leq 0.05$ on two-tailed (Suhartono et al. 2021).

RESULTS AND DISCUSSION

During this study, 129 isolates of *Klebsiella pneumoniae* of variable colony color were observed from sputum on three different media, i.e., MacConkey Agar, Blood Agar, and Chocolate Agar (Figure 1). *K. pneumoniae* colonies had round, pink, slippery edges, convex elevation, and a 2 to 5 mm diameter on MacConkey Agar. On both Blood Agar and Chocolate Agar, *K. pneumoniae* colonies appeared round, grayish-white without any clear hemolytic zones, with slippery edges, convex elevation, and a size ranging from 2-5 mm. Gram staining of *K. pneumoniae* isolates obtained from sputum specimens exhibiting typical morphology Gram-negative short rods characteristic (Figure 2).

Of 129 isolates, the distribution of ESBL-producing and non-ESBL-producing *K. pneumoniae* in sputum specimens for the period January 2021 to January 2024 was 103 (79.84%) and 26 (20.16%), respectively (Table 1). The test revealed no statistically significant correlation between the distribution of (χ^2=4.9257, p=0.0852, significance level=0.05) ESBL and non-ESBL-producing *K.*

pneumoniae isolates across the three sample collection periods. The prevalence of ESBL-producing *K. pneumoniae* increased over the three periods, from 24 isolates in Period I (January 2021-January 2022) to 53 isolates in Period III (February 2023-January 2024), while the number of non-ESBL-producing isolates remained relatively stable.

Figure 3 shows the frequency (%) distribution of ESBL-producing and non-ESBL *K. pneumoniae* isolates recovered from sputum specimens. Based on the statistical analysis, there was no significant association between the number of males and the number of females distributing the isolates *K. pneumoniae* (χ^2=0.0186, p=0.8914, significance level=0.05). This is evident from the percentage of ESBL and non-ESBL isolates of *K. pneumoniae* are found to be very close to each other in both males and females. However, the table shows a striking difference when comparing the overall prevalence between males and females. The frequency (%) distributions of ESBL-producing and non-ESBL *K. pneumoniae* are highest in males (67.96 and 62.23, respectively) compared to females (32.04 and 30.77, respectively).

The frequency distribution of ESBL-producing and non-ESBL-producing *K. pneumoniae* isolates from sputum specimens based on age groups (0-5, 6-11, 12-25, 26-45, and >45) at Zainoel Abidin General Hospital for January 2021 to January 2024 is presented in Figure 4. The statistical test revealed no statistically significant correlation between the distribution of *K. pneumoniae* isolates and age groups (χ^2=7.5855, p=0.1079, significance level=0.05). This suggests that the proportions of ESBL and non-ESBL isolates are similar across the different age groups. However, Figure 4 also highlights differences in the overall prevalence of *K. pneumoniae* isolates among the age groups. The frequency distribution of ESBL-producing and non-ESBL-producing *K. pneumoniae* was highest in the >45 age group (81.55 and 76.92%, respectively).

The frequency distribution of ESBL-producing and non-ESBL-producing *K. pneumoniae* isolates from sputum specimens based on hospital wards at Zainoel Abidin General Hospital for January 2021 to January 2024 is presented in Figure 5. A chi-square test for independence was performed to determine if there is a significant association between the type of isolate (ESBL or non-ESBL) and the hospital ward. The test revealed no statistically significant correlation between the distribution of *K. pneumoniae* isolates and hospital wards (χ^2=18.8958, p=0.1266, significance level=0.05). This suggests that the proportions of ESBL and non-ESBL isolates are similar across the different hospital wards.

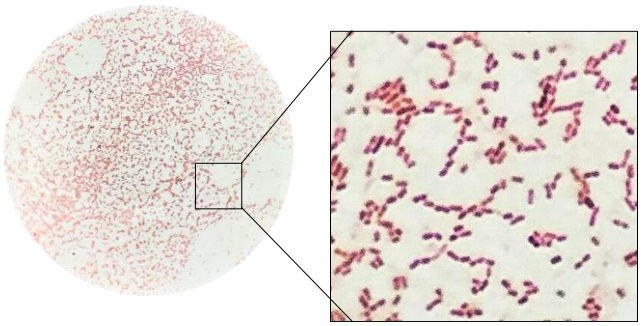

Figure 2. Gram staining of *Klebsiella pneumoniae* isolated from sputum specimens, showing characteristic Gram-negative short rods with magnification 1000×

Table 1. Number of ESBL-producing and non-ESBL-producing *Klebsiella pneumoniae* isolates recovered from sputum specimens at Zainoel Abidin General Hospital from January 2021 to January 2024. Based on the Chi-square test for independence test, the type of isolates and the periods were independent (χ^2=4.9257, p=0.0852)

***Klebsiella pneumoniae* phenotype**	**Periods**			**Total**
	I January 2021-January 2022	**II February 2022-January 2023**	**III February 2023-January 2024**	
ESBL	24	26	53	103(79.84%)
Non-ESBL	6	11	9	26(20.16%)
Total	30	37	62	129(100%)

A **B** **C**

Figure 1. Growth of *Klebsiella pneumoniae* colonies on A. MacConkey Agar; B. Blood Agar; and C. Chocolate Agar after 24 hours of incubation at 37°C. Characteristic mucoid pink colonies were observed on MacConkey Agar, while grayish-white colonies without hemolysis were seen on Blood Agar and Chocolate Agar

However, Figure 5 also highlights differences in the overall prevalence of *K. pneumoniae* isolates in hospital wards. The highest frequency distribution of *K. pneumoniae* (n=129) was observed in the heart and lung rooms at 25/129. In the heart and lung rooms, ESBL-producing *K. pneumoniae* (n=103) was observed at 15/103, whereas the highest frequency distribution of non-ESBL-producing *K. pneumoniae* (n=26) was also found in the heart and lung rooms (10/26).

In terms of antibiotic susceptibility, for the ESBL-producing *K. pneumoniae* isolates, the highest susceptibility was observed for amikacin (92.23%), followed by doripenem (89.32%), meropenem (87.38%), imipenem (86.41%), and cefoperazone/sulbactam (85.52%) (Table 2). These isolates showed high resistance to amoxicillin (0% susceptibility), cefotaxime (1.94%), ceftriaxone (1.94%), cefoxitin (20.39%), and ceftazidime (23.3%). Moderate susceptibility was seen for gentamicin (57.28%), tobramycin (65.05%), levofloxacin (51.46%), and doxycycline (51.46%). In contrast, the non-ESBL-producing *K. pneumoniae* isolates demonstrated 100% susceptibility to cefotaxime, ceftriaxone, amikacin, and tobramycin. High susceptibility was also observed for cefoxitin (92.31%), ceftazidime (92.31%), gentamicin (96.15%), levofloxacin (92.31%), and doxycycline (76.92%). Like the ESBL-producing isolates, non-ESBL-producing *K. pneumoniae* showed complete resistance to amoxicillin (0% susceptibility) and low susceptibility to ampicillin (3.85%). The chi-square test revealed statistically significant differences ($p<0.05$) in susceptibility between ESBL-producing and non-ESBL-producing *K. pneumoniae* for several antibiotics, including cefoxitin, cefotaxime, ceftazidime, ceftriaxone, gentamicin, tobramycin, levofloxacin, and doxycycline. This indicates that ESBL production significantly impacts the antibiotic susceptibility profile of *K. pneumoniae* isolates.

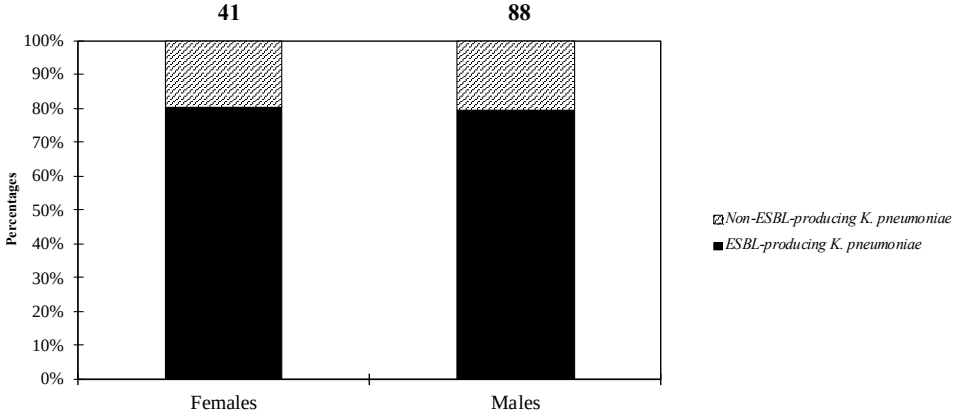

Figure 3. Frequency of occurrence (%) ESBL-producing *Klebsiella pneumoniae* (n=103) and non-ESBL-producing *K. pneumoniae* (n=26) in sputum specimens based on gender at Zainoel Abidin General Hospital for the period January 2021 to January 2024. The numbers above each column are the total number of isolates recovered from each gender. Based on the Chi-square test for independence test, the isolates and the patients' gender were independent (χ^2=0.0186, p=0.8914)

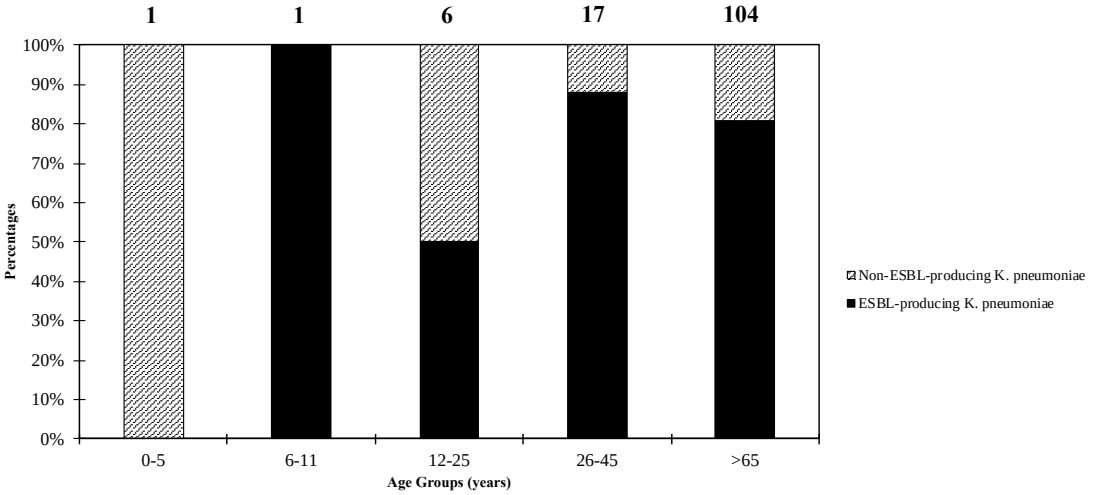

Figure 4. Frequency of occurrence (%) ESBL-producing *Klebsiella pneumoniae* (n=103) and non-ESBL-producing *K. pneumoniae* (n=26) in sputum specimens based on age groups at Zainoel Abidin General Hospital for the period January 2021 to January 2024. The numbers above each column are the total number of isolates recovered from each gender. Based on the Chi-square for independence test, the isolates and the patients' age groups were independent (χ^2=7.5887, p=0.1079)

Table 2. Antibiotic susceptibility of ESBL-producing *Klebsiella pneumoniae* (n=103) and non-ESBL-producing *K. pneumoniae* (n=26) in sputum specimens based on patients' wards at Zainoel Abidin General Hospital from January 2021 to January 2024

Antibiotics	ESBL-producing *K. pneumoniae* (n=103)		non-ESBL-producing *K. pneumoniae* (n=26)		Chi-2[a]	*p-values*[b]
	n	%	n	%		
Amoxicillin	0	0	0	0	n.a	n.a
Ampicillin	1	0.97	0	0	0.2540	0.6143
Amoxicillin/Clavulanic	82	79.61	25	96.15	4.2087	0.0402
Piperacillin/Tazobactam	75	72.82	23	88.46	2.8472	0.1175
Cefoxitin	21	20.39	24	92.31	53.9515	< 0,001
Cefotaxime	2	1.94	26	100	117.3636	< 0,001
Ceftazidime	24	23.3	24	92.31	42.5000	< 0,001
Ceftriaxone	2	1.94	26	100	117.3636	< 0,001
Cefoperazone/Sulbactam	85	85.52	25	96.15	3.0706	0.0797
Doripenem	92	89.32	25	96.15	1.1285	0.6891
Imipenem	89	86.41	24	92.31	0.6648	0.4149
Meropenem	90	87.38	24	92.31	0.4922	0.4829
Amikacin	95	92.23	26	100	2.1936	0.2059
Gentamicin	59	57.28	25	96.15	13.8000	0.0002
Tobramycin	67	65.05	26	100	12.5405	0.0003
Levofloxacin	53	51.46	24	92.31	14.3200	0.0004
Doxycycline	53	51.46	20	76.92	5.4576	0.0195

Notes: [a] Chi-square calculated for comparison of susceptibility in ESBL-producing *K. pneumoniae* and non-ESBL-producing *K. pneumoniae*; [b] P value generated from the chi-square

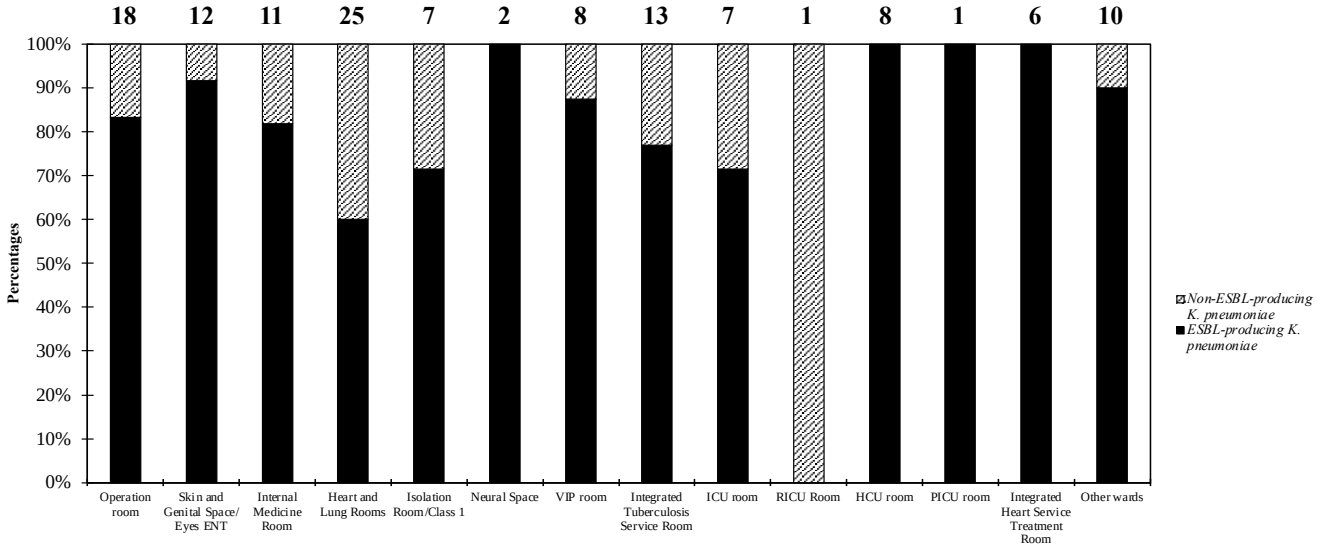

Figure 5. Frequency of occurrence (%) ESBL-producing *Klebsiella pneumoniae* (n=103) and non-ESBL-producing *K. pneumoniae* (n=26) in sputum specimens based on patients' wards at Zainoel Abidin General Hospital for the period January 2021 to January 2024. The numbers above each column are the total number of isolates recovered from each gender. Based on the Chi-square test for independence test, the isolates and the patients' age groups were independent (χ^2=18.8958, p=0.1266)

Discussion

In the present study, 129 *K. pneumoniae* isolates were recovered from sputum specimens, providing useful insights into the incidence and attributes of this clinically relevant pathogen within hospital settings. Based on the consistent morphologies in these bacterial isolates, including the pink, mucoid colonies on MacConkey agar for taxonomic characteristics indicates the fermentation of lactose gut sugars in the plate (Bolla et al. 2021); grayish-white, non-hemolytic on blood and chocolate agar (Bolla et al. 2021; Abdurahman and Musa 2022) are typical characteristics for *K. pneumoniae*. The microscopy observed the short rod shape of the bacterial isolates, which verifies the morphological features of *K. pneumoniae*. The lack of other morphological forms indicates that the cultures are pure isolates of *K. pneumoniae* (Zhu et al. 2021). Gram staining and selective media (MacConkey Agar, Blood Agar, and Chocolate Agar) were conducted on the *K. pneumoniae* as this approach offered faster, more reliable, and more cost-effective that might be required for timely decisions in the clinical lab. Furthermore, these techniques are less costly than some biochemical tests,

making them affordable for routine use in several labs. The combination of Gram staining and selective media has been accepted for the preliminary identification of *K. pneumoniae*, and they provide reliable results that could be confirmed by more specific tests if necessary (Suhartono et al. 2021).

The high prevalence of extended-spectrum beta-lactamase (ESBL)–producing *K. pneumoniae* (79.84%) mirrors the rising trend of antibiotic resistance among *Enterobacteriaceae* across the globe (Suhartono et al. 2021; Siriphap et al. 2022). The increasing trend in the prevalence of ESBL producers during the study period (Ahmad et al. 2021; Hayati et al. 2021) is particularly concerning for escalating resistance in the hospital setting. This signifies the prevalence of ESBL-producing *K. pneumoniae* is higher than observed in some previous studies (Anggraini et al. 2017; Sinanjung et al. 2020) conducted in Indonesia. Therefore, it can be hypothesized that the higher prevalence of ESBL producers might be attributed to certain hospital-level factors in our study settings, including but not limited to antibiotics, infection control practices, and patient characteristics (Al-Garni et al. 2018). Our findings from a single tertiary care hospital in Banda Aceh, Indonesia, may not represent ESBL distribution in other healthcare settings or geographic areas. The prevalence and distribution of ESBL-producing *K. pneumoniae* can vary significantly due to differences in antibiotic use patterns, infection control practices, patient populations, and local epidemiology. Future studies should aim to correlate ESBL prevalence with antibiotic consumption data and assess the implementation of infection control measures to provide a more comprehensive understanding of the factors driving ESBL emergence and spread in this setting.

Demographic, sex, and age comparison showed that male patients and those over 45 were more frequently reported as infected with ESBL-producers or non-ESBL-producers *K pneumoniae*. These differences were probably related to the biological and environmental risk factors for *K pneumoniae* infection, such as possible sex-dependent anatomical differences, sex hormones, or different exposures to risk factors, including systemic catheterization, previous medical interventions, or hospitalization (Chang et al. 2021; Dias et al. 2022). While our study focused on the age and sex distribution of ESBL and non-ESBL *K. pneumoniae*, we recognize that environmental factors and patient medical history likely play significant roles in the epidemiology of these infections. Future studies should aim to collect and analyze data on environmental contamination, antibiotic exposure history, previous hospitalizations, and underlying comorbidities to provide a more comprehensive understanding of the factors influencing ESBL and non-ESBL *K. pneumoniae* distribution in our setting. Identifying these risk and environmental factors could improve the epidemiological understanding of *K pneumoniae* infection, especially the ESBL-producers.

A spatial distribution of *K. pneumoniae* isolates (ESBL- and non-ESBL-producing isolates) in the wards revealed an uneven distribution, and the types of isolates have no correlation within hospitals: ESBL-producing isolates were more likely than non-ESBL producers to exist in surgical and cardiopulmonary wards; non-ESBL-producing isolates were more prevalent in the cardiopulmonary wards. This distribution pattern is particularly significant as it suggests that mechanical ventilation and endotracheal intubation remain a blind spot for nursing care during the treatment, highlighting extra sources (rather than direct cross-infections) and tend to be nosocomial transmission. *K. pneumoniae* is the major common cause of community-acquired respiratory tract infections, and colonized patients and carriers are more frequent in the cardiopulmonary wards (Meng et al. 2020).

Antibiotic susceptibility testing revealed a clear divergence in resistance patterns between ESBL-producing and non-ESBL-producing *K. pneumoniae*. Unsurprisingly, ESBL producers displayed increased resistance to several beta-lactamases as ESBL enzymes hydrolyze these drugs (Šuto et al. 2022). This demonstrates why accurately identifying ESBL-producing strains is important to ensure appropriate antibiotic therapy and prevent treatment failure. Interestingly, non-ESBL-producing isolates were mostly susceptible to most antibiotics tested, except amoxicillin and ampicillin, which could be very useful when keeping as many treatment options open as possible. Importantly, even some ESBL-producing isolates demonstrated susceptibility to amikacin, largely because aminoglycosides such as amikacin are resistant to ESBL hydrolysis (Garneau-Tsodikova and Labby 2016). This finding suggests that those producing ESBLs can still survive when beta-lactam antibiotics are ineffective. Similarly, carbapenems and cefoperazone/sulbactam also demonstrated activity against ESBL producers, which is helpful as more and more isolates develop resistance to the increasing arsenal of anti-ESBL drugs that have been introduced in the last decade. Such differences in susceptibility can be useful for tracking antibiotic resistance over time to decide what's worth stockpiling for medicine cabinets or can be used to design a useful antibiotic stewardship program.

This suggests that with the increasingly diversified antimicrobial resistance profiles of ESBL-producing *K. pneumoniae*, this pathogen will pose a challenging issue for our hospital and the wider healthcare community. Continued monitoring, strict infection control measures, and appropriate antibiotic stewardship (Ahmad et al. 2021; Suhartono et al. 2021) are essential in the increasing peril of antibiotic resistance and ensuring patients' health. Our study provides valuable insights for clinicians in the region to choose judicious antibiotics and empirical antibiotic therapy to deal with the local epidemiology and antibiotic susceptibility of ESBL-producing *K. pneumoniae*.

Although this study could fill a void in knowledge about prevalence and resistance among ESBL-producing and non-ESBL-producing *K. pneumoniae*, some limitations exist. Our study was conducted at a single hospital, which may limit the generalizability of the findings. The prevalence of ESBL-producing *K. pneumoniae* may be influenced by local factors such as antimicrobial stewardship programs and infection control practices,

which were not assessed in this study. Furthermore, the sample size may be limited for finding the true diversity of *K. pneumoniae* strains worldwide that have not been associated with a certain region or specific healthcare facility (Hayati et al. 2021). The seasonal sampling lasted for only three years, which is quite a short period to follow seasonal or long-term trends in antibiotic resistance. Longer studies would be helpful to more fully unravel the emerging patterns of resistance (Ahmad et al. 2021). Additionally, although Gram staining and selective media are useful for presumptive identification, the study did not use molecular methods for confirmation, which could help concretize the results (Shrief et al. 2022). Moreover, the study did not elucidate the genotypic basis of resistance, which could reveal more about the mechanisms contributing to ESBL production (Jiang et al. 2020). Finally, the study did not put the findings into the medical context by exploring the clinical outcomes of the patients or the risk factors for acquiring ESBL-producing *K. pneumoniae* infections, which can help to evaluate the clinical impact of antibiotic resistance and tailor targeted interventions (Chang et al. 2021).

In conclusion, the research on *K. pneumoniae* isolated from sputum specimens at Zainoel Abidin General Hospital Banda Aceh, Indonesia, underscores a worrying trend of increasing antibiotic resistance, particularly with ESBL-producing strains. These findings underscore the urgent need for stricter infection control measures to prevent the spread of antibiotic resistance. The largely descriptive nature of this study significantly contributes to our understanding of the local epidemiology and susceptibility patterns of ESBL-producing *K. pneumoniae*, aiding stakeholders in addressing the formidable challenge of antimicrobial resistance that threatens millions of lives worldwide.

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