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Risk factors for acquisition of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* on non-ventilator-associated hospital-acquired pneumonia in a tertiary care hospital in Indonesia

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ABSTRACT

Aims: This study was aimed to identify the risk factors for the acquisition of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* and *Klebsiella pneumoniae* on non-ventilator hospital-acquired pneumonia (NV-HAP) patients in a tertiary care hospital in Indonesia.
Methodology and results: A case-control study was performed between March 31, 2018, and August 31, 2019. Twenty-eight ESBL-producing *E. coli* and *K. pneumoniae* isolates and 28 susceptible strains of *E. coli* and *K. pneumoniae* obtained from NV-HAP patients were included in this study. Phenotypic screening for ESBL production was performed by the Vitek2 system and subsequently confirmed by double-disk synergy tests. The use of 3rd generation cephalosporin as initial antibiotic therapy for more than three days was the significant risk factor for the acquisition of ESBL-producing *E. coli* and *K. pneumoniae* among NV-HAP patients (odds ratio [OR] 41.827; p=0.001). The length of stay of patients with NV-HAP acquiring the ESBL strains was longer than 10 days (OR 17.334; p=0.001).
Conclusion, significance and impact of study: The use of 3rd generation cephalosporin as the initial antibiotic for NV-HAP should be restricted to prevent the emergence of ESBL-producing strains. Infection prevention measures are required to control the acquisition of ESBL-producing *E. coli* and *K. pneumoniae* in NV-HAP patients.

Keywords: ESBL, *Escherichia coli*, Indonesia, *Klebsiella pneumonia*, non-ventilator hospital-acquired pneumonia (NV-HAP)

INTRODUCTION

Hospital-acquired pneumonia (HAP) is a critical healthcare-associated infection leading to high morbidity and mortality worldwide (Di Pasquale *et al*., 2016). Davis and Finley (2012) reported that non-ventilator-associated HAP (NV-HAP) was more predominant than ventilator-associated pneumonia (VAP) with the same significant impact as VAP; however, studies on NV-HAP are scarce (Davis and Finley, 2012; Mitchell *et al*., 2019). The pathogens causing NV-HAP vary depending on pneumonia onset, comorbidities and prior antibiotic therapy (Di Pasquale *et al*., 2016). According to previous studies in the United States and European countries, *Escherichia coli* and *Klebsiella pneumoniae* are consistently the most frequently found etiologic agent causing HAP since 1985 (Jones, 2010; Luyt *et al*., 2018). *Escherichia coli* and *K. pneumoniae* were the most prevalent Enterobacteriaceae isolates obtained from sputum culture in Indonesian hospitals in 2020 (Anggraini, 2021). The emergence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and *K. pneumoniae* complicates antibiotic therapy against those multidrug-resistant organisms (MDRO). The ESBLs are capable of presenting bacterial resistance to penicillin, broad-spectrum cephalosporins and monobactams by hydrolysis of these antibiotics, but their activities are inhibited by clavulanic acid (Abayneh *et al*., 2018). Beta-lactamases are classified based on the Ambler molecular classification scheme into class A, class B, class C and class D. The other classification system is according to the Bush-Jacoby-Medeiros classification scheme based on the functional similarities (Paterson and Bonomo, 2005).

ESBL-producing *E. coli* and *K. pneumoniae* are the significant pathogens causing NV-HAP, which has been

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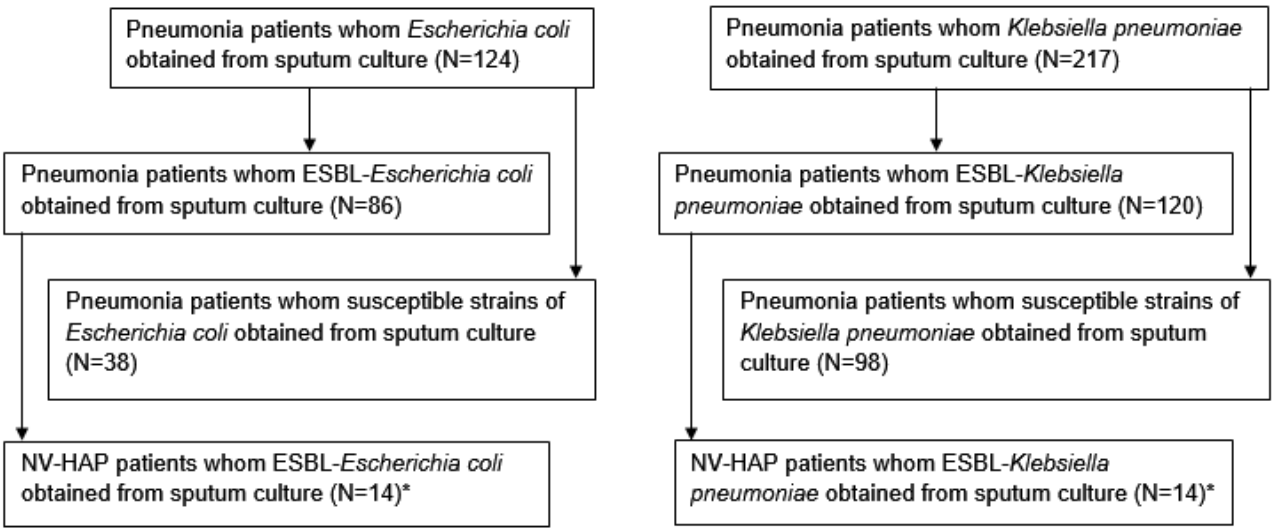

Figure 1: Numbers of ESBL-producing *E. coli* and *Klebsiella pneumoniae* were obtained from adult patients involved in this study (NV-HAP=non-ventilator associated-hospital acquired pneumonia; ESBL=extended spectrum beta-lactamase; *detected by DDST).

often associated with mortality, prolonged hospital stays and cost, particularly in developing countries (Abayneh *et al*., 2018; Liu *et al*., 2019). The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* from sputum culture in Indonesian hospitals was 71% and 46%, respectively (Anggraini, 2021).

Colonization of MDRO is one of the risk factors for MDRO infections (Ridgway *et al*., 2013). Therefore, knowledge of the risk factors for ESBL-producers acquisition among NV-HAP patients is needed for the implementation of infection control measures in hospitals to improve the clinical outcome. In addition, better identification of NV-HAP patients at high-risk infection caused by ESBL-producing bacteria is vital to refining the therapeutic approach for proper selection of the initial antibiotic therapy (Razazi *et al*., 2017; Luyt *et al*., 2018). Controlling the ESBL infections might reduce the use of carbapenem antibiotics and carbapenem resistance subsequently. However, little is known about the risk factors for ESBL-producers acquisition, particularly among NV-HAP patients in healthcare settings in Indonesia. This study aimed to analyze the risk factors for the acquisition of ESBL-producing *E. coli* and *Klebsiella pneumoniae* among NV-HAP patients in a resource-limited hospital in Indonesia.

MATERIALS AND METHODS

Study design

We performed a case-control study including 28 NV-HAP patients acquiring ESBL-producing *E. coli* and *K. pneumoniae* and 28 NV-HAP patients who acquired susceptible strains of *E. coli* and *K. pneumoniae* in Dr. Saiful Anwar Hospital (tertiary care hospital; 882 beds), Malang, Indonesia. The data was collected between March 2018 and August 2019 prospectively. Only the first isolate of either *E. coli* or *K. pneumoniae* obtained from a non-invasive respiratory sample per patient in the internal medicine wards was included in this study and analyzed anonymously. The study was approved by the medical ethics committee of Dr. Saiful Anwar Hospital (No:400/022/K.3/302/2019).

Definitions

The diagnosis of NV-HAP was defined as an episode of pneumonia that was not associated with mechanical ventilation, occurring more than 48 h after admission and not intubated at hospital admission (Giuliano *et al*., 2018). A case was defined as an NV-HAP patient from whom either ESBL-producing *E. coli* or *K. pneumoniae* could be isolated from a non-invasive respiratory sample. A control was defined as a NV-HAP patient from whom susceptible strains either *E. coli* or *K. pneumoniae* were isolated from a non-invasive respiratory sample. However, NV-HAP patients with incomplete data in the medical records were excluded. The number of enrolled and analyzed subject is presented in Figure 1.

Microbiological examination

Sputum cultures were performed according to the routine diagnostic testing in the Laboratory of Clinical Microbiology in Dr. Saiful Anwar Hospital, Malang, Indonesia. *Escherichia coli* and *K. pneumoniae* isolates were identified and screened for ESBL-producing strains using the Vitek2 system (bioMérieux). Confirmation of suspected ESBLs producers was conducted using a double-disk synergy test (DDST) on Mueller Hinton agar (Drieux *et al*., 2008; Abayneh *et al*., 2018). Briefly, a disk of amoxicillin/clavulanic acid (20 µg/10 µg) was placed

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Table 1: Univariate analysis of risk factors for NV-HAP patients acquiring ESBL-producing *E. coli* and *Klebsiella pneumoniae* in a resource-limited hospital in Indonesia.

Variables	Number of subjects (%)			
	Total (n=56)	Non ESBL-producers (n=28)	ESBL-producers (n=28)	*p* values
Male, gender	29 (51.8)	14 (50)	15 (53.6)	0.500
Age, years	56.8 ± 16.9	59.4 ± 14.0	54.2 ± 19.3	0.259
Charlson comorbitidy index	1.7 ± 1.8	1.0 ± 1.4	2.5 ± 1.8	0.001*
Prior hospitalization	39 (69.6)	20 (71.4)	19 (67.9)	0.500
Prior use of the 3rd generation cephalosporins	49 (87.5)	25 (89.3)	24 (85.7)	0.500
Duration of the 3rd generation cephalosoporins use	5.5 ± 4.6	3.2 ± 3.2	7.8 ± 4.7	<0.001*
Length of stay (days)	12.7 ± 9.3	7.3 ± 4.3	18.2 ± 9.8	<0.001

NV-HAP=non ventilator associated-hospital acquired pneumonia; ESBL=extended spectrum beta lactamase; *data are presented as mean ± standard deviation.

between the cefotaxime (30 µg) and ceftazidime (30 µg) disks at a distance of 20 mm (center to center). A clear extension of the edge of the inhibition zone of cephalosporin towards the amoxicillin/clavulanic acid disk is interpreted as positive for ESBL production (Drieux *et al*., 2008; Rawat and Nair, 2010).

Clinical database

Basic clinical characteristics include age, gender, prior of hospitalization, the use of 3rd generation of cephalosporins upon admission, the duration of 3rd generation of cephalosporins use during hospitalization, comorbidities (diabetes mellitus, chronic kidney disease [CKD] and cerebrovascular disease [CVA]) and length of stay. The clinical database was obtained from the medical records. The points of the Charlson comorbidity index were used to calculate the severity of comorbidities.

Statistical analysis

Statistical analysis was performed using SPSS version 22.0. An independent *t*-test was used to assess continuous variables. Categorical variables were analyzed using Fisher's exact test. All categorical variables with a *p*-value less than 0.2 were included in a multivariate logistic regression model. Backward selection based on the likelihood-ratio test was used to identify significant variables. A *p*-value less than 0.05 was considered significant.

RESULTS

A total of 341 isolates consisting of *E. coli* (n=124) and *K. pneumoniae* (n=217) were obtained from sputum cultures during the study period. Of these, 69.4% (n=86) and 55.2% (n=120) were ESBL-producing *E. coli* and *K. pneumoniae*, respectively. Twenty-eight ESBL-producer strains from NV-HAP patients, including 14 ESBL-producing *E. coli* isolates and 14 ESBL-producing *K. pneumoniae* isolates were included in the risk factors analysis. Moreover, twenty-eight non-ESBL-producer strains from NV-HAP patients, including 8 non-ESBL-producing *E. coli* isolates and 20 non-ESBL-producing *K. pneumoniae* isolates were included in the control group.

Univariate analysis showed the association between NV-HAP patients acquiring ESBL-producing *E. coli* and *K. pneumoniae* with the duration of 3rd generation cephalosporins use during hospitalization (*p*=0.001), comorbidities severity level (*p*=0.005) and length of stay (*p*<0.001) (Table 1). Multiple logistic regression analysis showed that the significant risk factors for NV-HAP patients with ESBL-producing *E. coli* and *K. pneumoniae* were the use of 3rd generation of cephalosporin for more than three days (odds ratio [OR] 41.827, 95% confidence interval [$CI_{95\%}$] 4.195-417.056; *p*=0.001) and length of stay longer than 10 days (OR 21.084, $CI_{95\%}$ 1.946-88.387; *p*=0.012) (Table 2).

DISCUSSION

This study is the first risk factors analysis for ESBL-producing *E. coli* and *K. pneumoniae* acquisition among NV-HAP patients in a resource-limited hospital in Indonesia. We reported that the use of 3rd generation cephalosporins for more than three days and length of stay of more than 10 days were the significant risk factors associated with the acquisition of ESBL-producing *E. coli* and *K. pneumoniae* among NV-HAP patients. The previous studies showed the association between prior antibiotics use and ESBL infections; however, the duration of the antibiotics use particularly 3rd generation of cephalosporins among NV-HAP patients was not described clearly (Tham *et al*., 2013; Shaikh *et al*., 2015; Kalluru *et al*., 2018; Chen *et al*., 2020; Lin *et al*., 2021). Ceftriaxone, a 3rd generation of cephalosporin, is the most common empirical antibiotic therapy used in Indonesian hospitals (Farida *et al*., 2017; Zavira *et al*., 2021). Our study suggested that the use of ceftriaxone as initial antibiotic therapy should be restricted to prevent the burden of ESBL-producer infections. Therefore, the antibiotic susceptibility test of sputum culture should be reported to the clinicians within 72 h to determine definitive antibiotic therapy.

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Table 2: Multivariate analysis of risk factors for NV-HAP patients acquiring ESBL-producing *E. coli* and *Klebsiella pneumoniae* in a resource-limited hospital in Indonesia.

Variables	Odds Ratio	95% Confidence Interval	*p* values
Age group:			0.500
< 60 years			
≥ 60 years			
Charlson comorbidity index:			0.998
0-3			
4-5			
Duration of the 3rd generation of cephalosporins use:	41.827	4.195-417.056	0.001
< 3 days			
≥ 3 days			
Length of stay:	17.334	3.400-88.387	<0.001
< 10 days			
≥ 10 days			

NV-HAP= non-ventilator associated-hospital acquired pneumonia; ESBL=extended spectrum beta-lactamase.

The comorbidities severity level was significantly higher among NV-HAP acquiring ESBL-producers than in the control group. CKD, CVA and diabetes mellitus are the underlying diseases calculated using the Charlson comorbidity index in this study. NV-HAP patients with more than one of the underlying diseases were at risk of acquiring ESBL-producing *E. coli* and *K. pneumoniae* during hospitalization. Other similar studies reported the coherent finding that patients with such underlying diseases were at risk for MDRO colonization (Santosaningsih *et al*., 2017; Rattanaumpawan *et al*., 2018).

NV-HAP patients staying in the hospital longer than 10 days were 17 times more likely to acquire ESBL-producing *E. coli* and *K. pneumoniae* in this study than the control group. Previous studies reported that prolonged hospital stay was a greater risk for MDRO acquisition (Tham *et al*., 2013; Chen *et al*., 2020). The longer length of stay could represent the worse clinical outcome that might be associated with MDRO infections.

This study has some limitations. First, we did not ascertain that either the ESBL-producing *E. coli* or *K. pneumoniae* isolated from the sputum culture was acquired during their hospital stay because patients may have been unrecognized carriers before admission to the hospital (Tham *et al*., 2013). Second, we analyzed the risk factors of underlying diseases by combining either CKD or CVA or diabetes mellitus; therefore, the association between each underlying disease and acquisition of ESBL-producing *E. coli* and *K. pneumoniae* among NV-HAP patients were not identified. Third, there was a small number of patients involved in this study.

CONCLUSION

In conclusion, NV-HAP patients who received the 3rd generation of cephalosporin for more than 3 days and stayed in the hospital longer than 10 days may be at risk of acquiring ESBL-producing *E. coli* and *K. pneumoniae*. The hospital antibiotic use policy is required to review the use of 3rd generation of cephalosporin, particularly ceftriaxone. The early detection of ESBL-producers infection and colonization is essential for infection prevention control purposes. Similar studies with more samples should be conducted in other referral hospitals in Indonesia to identify additional risk factors for NV-HAP patients that were not detected in this study.

CONFLICT OF INTEREST

All authors report no conflicts of interest relevant to this article.

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