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 *Escherichia 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*).

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 (Dneux et al., 2006; Abayneh et al., 2018). Briefly, a disk of amoxicillin/clavulanic acid (20 µg/10 µg) was placed...
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 [CI95%] 4.195-417.056; p=0.001) and length of stay longer than 10 days (OR 21.084, CI95% 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 longer 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.
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.

<table>
<thead>
<tr>
<th>Variables</th>
<th>Odds Ratio</th>
<th>95% Confidence Interval</th>
<th>p values</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age group:</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 60 years</td>
<td></td>
<td></td>
<td>0.500</td>
</tr>
<tr>
<td>≥ 60 years</td>
<td></td>
<td></td>
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<tr>
<td>Charlson comorbidity index:</td>
<td></td>
<td></td>
<td>0.998</td>
</tr>
<tr>
<td>0-3</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4-5</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Duration of the 3rd generation of cephalosporins use:</td>
<td>41.827</td>
<td>4.195-417.056</td>
<td>0.001</td>
</tr>
<tr>
<td>&lt; 3 days</td>
<td></td>
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<td></td>
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<tr>
<td>≥ 3 days</td>
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<td></td>
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<tr>
<td>Length of stay:</td>
<td></td>
<td></td>
<td>&lt;0.001</td>
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<tr>
<td>&lt; 10 days</td>
<td>17.334</td>
<td>3.400-88.387</td>
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<tr>
<td>≥ 10 days</td>
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</table>

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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