



JMedSci

## Extended spectrum beta lactamase (ESBL)-producing *Klebsiella pneumoniae* clinical isolates and its susceptibility pattern to antibiotics at Dr. Soeradji Tirtonegoro General Hospital Klaten, Central Java

Kian Sinanjung<sup>1</sup>, Abu Tholib Aman<sup>2</sup>, Hera Nirwati<sup>2\*</sup>

<sup>1</sup>Resident of Clinical Microbiology, Faculty of Medicine, Public Health, and Nursing Universitas Gadjah Mada. <sup>2</sup>Department of Microbiology, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia

### ABSTRACT

Submitted: 2019-09-01  
Accepted : 2019-09-19

Globally, the prevalence of *Klebsiella pneumoniae* (*K. pneumoniae*) producing extended spectrum beta lactamase (ESBL) has been increasing steadily. The susceptibility patterns of ESBL-producing *K. pneumoniae* varies considerably among countries. Therefore, the investigation of ESBL-producing *K. pneumoniae* in clinical isolates and their susceptibility are warranted. This research aimed to determine the proportion of ESBL-producing *K. pneumoniae* and the antibiotic susceptibility patterns of clinical isolates from Dr. Soeradji Tirtonegoro General Hospital, Klaten, Central Java. Identification of *K. pneumoniae* was performed by analyzing colony morphology, microscopic examination, and biochemical testing using Microbact. Both antibiotic susceptibility testing and ESBL screening (using ceftazidime, cefotaxime, and ceftriaxone discs) were conducted using disc diffusion method according to CLSI. The positive results were confirmed with modified double disk synergy (MDDST) using amoxicillin-clavulanate, ceftazidime, cefotaxime, and cefepime discs. From 962 clinical bacterial isolates, 168 (17.46%) isolates were identified as *K. pneumoniae*, during June 2017-May 2018. *K. pneumoniae* was mainly isolated from the Intensive Care Units (ICU) (29.17%) and with sputum being the most common specimen (45.24%). Overall ESBL producers were 52.98%, with the majority from ICU (41.57%) and isolated from sputum specimens (40.45%). ESBL-producing *K. pneumoniae* showed high resistance to many antibiotics. The sensitivity of ESBL-producing *K. pneumoniae* isolated from respiratory tract samples against piperacillin-tazobactam, amikacin, and meropenem was more than 80%. In conclusion, among all *K. pneumoniae* isolates, ESBL *K. pneumoniae* was 52.98%. ESBL *K. pneumoniae* from respiratory tract specimens had a sensitivity of more than 80% against piperacillin-tazobactam, amikacin, and meropenem.

### ABSTRAK

Prevalensi *Klebsiella pneumoniae* (*K. pneumoniae*) yang menghasilkan *extended spectrum beta lactamase* (ESBL) telah meningkat terus-menerus secara global. Pola kerentanan *K. pneumoniae* penghasil ESBL sangat bervariasi di antara negara-negara di dunia. Oleh karena itu diperlukan penyelidikan *K. pneumoniae* penghasil ESBL pada isolat klinis dan kerentanannya. Penelitian ini bertujuan untuk menentukan proporsi *K. pneumoniae* yang memproduksi ESBL dan pola kerentanan antibiotik isolat klinis dari Rumah Sakit Dr. Soeradji Tirtonegoro Klaten, Jawa Tengah. Identifikasi *K. pneumoniae* dilakukan dengan menganalisis morfologi koloni, pemeriksaan mikroskopis, dan pengujian biokimia menggunakan Microbact. Kedua uji kerentanan antibiotik dan skrining ESBL (menggunakan cakram ceftazidim, cefotaksim, dan ceftriaxone) dilakukan dengan menggunakan metode difusi cakram menurut CLSI. Hasil positif dikonfirmasi dengan *modified double disk synergy* (MDDST) menggunakan amoksisilin-klavulanat, ceftazidim, cefotaksim, dan cakram cefepim. Dari 962 isolat bakteri klinis, 168 (17,46%) isolat diidentifikasi sebagai

**Keywords:**

*Klebsiella pneumoniae*;  
extended spectrum beta  
lactamase;  
modified double disk  
synergy;  
antibiotic resistance;  
susceptibility testing;

*K. pneumoniae*, selama Juni 2017-Mei 2018. *K. pneumoniae* terutama diisolasi dari *intensive care unit* (ICU) (29,17%) dan dengan dahak sebagai spesimen umum (45,24%). Secara keseluruhan produsen ESBL adalah 52,98%, dengan mayoritas dari ICU (41,57%) dan diisolasi dari spesimen dahak (40,45%). *K. pneumoniae* yang memproduksi ESBL menunjukkan resistensi tinggi terhadap banyak antibiotik. *K. pneumoniae* penghasil ESBL yang diisolasi dari sampel saluran pernapasan menunjukkan sensitivitas terhadap piperasilin-tazobactam, amikasin, dan meropenem lebih dari 80%. Dapat disimpulkan di antara semua isolat *K. pneumoniae*, ESBL *K. pneumoniae* adalah 52,98%. ESBL *K. pneumoniae* dari spesimen saluran pernapasan memiliki sensitivitas lebih dari 80% terhadap piperasilin-tazobaktam, amikasin, dan meropenem.

## INTRODUCTION

*Klebsiella pneumoniae* is one of the bacteria with a high level of antibiotic resistance and often causes nosocomial infections in hospitals.<sup>1,2</sup> *Klebsiella pneumoniae* is known to cause pneumonia, urinary tract infection (UTI), bacteremia, liver abscess, wound infection, intravascular infection, bile duct infection, peritonitis, rhinoscleroma meningitis, ozaena, sinusitis, otitis, enteritis, appendicitis, cholecystitis, pyogenic brain abscess, and endophthalmitis.<sup>2-5</sup> It is often isolated from ICUs and associated with outbreaks in hospitals.<sup>6</sup>

Extended spectrum beta-lactamase (ESBL) is a mutated  $\beta$  lactamase enzyme capable of hydrolyzing penicillin, first, second, third and fourth generation cephalosporin, and monobactam but cannot hydrolyze cephamycins or carbapenem.<sup>7</sup> ESBL encoding genes are frequently found on the same plasmids that encode resistance to aminoglycosides, sulfonamides, and fluoroquinolones, so that ESBL-producing bacteria are commonly multidrug-resistant (MDR) bacteria.<sup>8</sup>

Various studies have shown an increased prevalence of ESBL-producing *K. pneumoniae* worldwide. Data from the tigecycline evaluation and surveillance trial (TEST) during January 2004 to August 2006 showed ESBL-producing *K. pneumoniae* in Latin America (44%), Asia (22.4%), Europe (13.3%), and North America (7.5%) respectively.<sup>9</sup> In China, ESBL-producing *K. pneumoniae*

was reported 31.8% of *K. pneumoniae* during August 2010-2011.<sup>10</sup> Meanwhile the prevalence of ESBL-producing *K. pneumoniae* in Iran was 43% of *K. pneumoniae* during 2007-2008<sup>11</sup> and in Tanzania it was 63.73% of *K. pneumoniae*.<sup>12</sup>

The prevalence of ESBL-producing *K. pneumoniae* in Indonesia was reported differently. At Dr. Soetomo General Hospital, Surabaya, the prevalence of ESBL-producing *K. pneumoniae* was reported 35.35% during January 2005-April 2005<sup>13</sup>; 23% during January-June 2010; 50.28% during January-November 2011; 58% during July-December 2012, and 38.5% during October 2014-May 2015.<sup>14,15</sup> Meanwhile the prevalence of ESBL-producing *K. pneumoniae* was reported 66.2% at Dr. Arifin Achmad General Hospital, Pekanbaru during January-December 2015.<sup>16</sup>

ESBL-producing bacteria are a major concern because they are associated with MDR microbes, which cause limited choices of antibiotics for the treatment of infections leading to increased morbidity, mortality and hospital costs.<sup>17</sup> Thus, the detection of ESBL-producing *K. pneumoniae* is important for optimal patient therapy choices and outcomes.

Screening of ESBL-producing *K. pneumoniae* can be done by disk diffusion using ceftazidime, cefotaxime, and ceftriaxone discs in accordance with the clinical laboratory standards institute (CLSI) M100 28<sup>th</sup> Edition.<sup>18</sup> This should be followed by a confirmation test with the modified double disk synergy test (MDDST) using ceftazidime, cefotaxime,

cefepime, and amoxicillin-clavulanate discs.<sup>19,20</sup>

The globally increasing prevalence of ESBL-producing *K. pneumoniae* infections, the increasing incidence of antibiotic resistance, and the increasing morbidity and mortality due to MDR bacterial infections in hospitals, prompt researchers to investigate ESBL-producing *K. pneumoniae* clinical isolates and their antibiotic sensitivity pattern. This research aimed to determine the proportion of ESBL-producing *K. pneumoniae* and the antibiotic susceptibility patterns of clinical isolates from Dr. Soeradji Tirtonegoro General Hospital, Klaten, Central Java.

## MATERIALS AND METHODS

### Study design and data collection

This was a cross-sectional descriptive study using secondary data from the archive of culture and susceptibility testing results at the Clinical Microbiology Laboratory Dr. Soeradji Tirtonegoro General Hospital, Klaten, Central Java during June 2017-May 2018. Screening and confirmation of ESBL-producing *K. pneumoniae* was conducted in the Microbiology Laboratory of the Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada, Yogyakarta, Indonesia. The research protocol was approved by The Medical and Health Research Ethics Committee, Faculty of Medicine, Public Health and Nursing, Universitas Gadjah Mada with number KE/FK/0784/EC/2018.

### Subjects

The subjects of the study were *K. pneumoniae* samples isolated from various clinical specimens of patients at Dr. Soeradji Tirtonegoro General Hospital, Klaten, Central Java during June 2017-May 2018.

### Isolation, identification, and antibiotic susceptibility test (AST)

Clinical specimens were inoculated on blood agar and Mac Conkey agar (Oxoid, UK). All incubations were conducted aerobically at 37<sup>o</sup> C for 18-24 hours.<sup>6</sup> Identification of *K. pneumoniae* isolates was based on the morphology of colonies on blood agar and Mac Conkey, microscopic examination with gram stain<sup>4,21</sup> and biochemical test using Microbact™ GNB 24E (Oxoid, UK). Biochemical tests were conducted according to company instructions.

*K. pneumoniae* isolates were tested for their AST by the disc diffusion method in accordance to CLSI guideline.<sup>18</sup> The following antibiotic discs from Oxoid, United Kingdom were used accordingly: cephazolin (30µg), gentamicin (10µg), tobramycin (10µg), amikacin (30µg), amoxicillin-clavulanate (20/10µg), piperacillin-tazobactam (100/10µg), cefuroxime (30µg), cefepime (30µg), ceftriaxone (30µg), ciprofloxacin (5µg), levofloxacin (5µg), meropenem (10µg), nitrofurantoin (100µg), trimethoprim-sulfamethoxazole (25µg), cefotaxime (30µg), and ceftazidime (30µg). Sensitive or resistant determination was based on CLSI references (18). Antibiotics were used according to the panel of agreement from the Indonesian Society for Clinical Microbiologist of 2015 based on CLSI references.

### Screening and confirmation of ESBL-producing *K. pneumoniae*

ESBL-producing *K. pneumoniae* screening was done with ceftriaxone, cefotaxime, and ceftazidime discs (Oxoid, UK) based on CLSI references.<sup>18</sup> The confirmation test of ESBL-producing *K. pneumoniae* was done using amoxicillin-clavulanate (20/10 µg) disc along with ceftazidime 30 µg, cefotaxime 30 µg, and cefepime 30 µg discs (Oxoid, UK). A lawn culture of the organisms was made

on a Mueller Hinton (MH) agar plate, as recommended by CLSI. Amoxicillin-clavulanate (20/10 µg) disc was placed in the center of the plate. The discs of ceftazidime 30 µg, cefotaxime 30 µg, and cefepime 30 µg were placed center to center to amoxicillin-clavulanate disc 20 mm apart. Then MH agar was incubated for 18-24 hours at 37°C. Any distortion or increase in the zone towards the disc of amoxicillin-clavulanate was considered as positive for the ESBL production.<sup>19,20</sup>

## RESULTS

During June 2017-May 2018, 168 (17.46%) of *K. pneumoniae* isolates were isolated from 962 total clinical bacterial isolates at Dr. Soeradji Tirtonegoro General Hospital Klaten, Central Java. The *K. pneumoniae* isolates were mainly obtained from patients treated in the intensive care unit (29.17%) (TABLE 1), and isolated from sputum specimens (45.24%) (TABLE 2).

TABLE 1. Specimen locations [n (%)] of *K. pneumoniae* isolates at Dr. Soeradji Tirtonegoro General Hospital, Klaten during June 2017-May 2018

Specimen locations	<i>K. pneumoniae</i> isolates (n=168)	ESBL producing <i>K. pneumoniae</i> isolates (n=89)
Intensives Care Unit	49 (29.17)	37 (41.57)
• Adults	27 (16.07)	16 (17.98)
• Children and Neonates	22 (13.10)	21(23.60)
Non-Surgery ward	37 (22.02)	10 (11.24)
Pulmonary ward	24 (14.29)	9 (10.11)
Surgery ward	23 (13.69)	11(12.36)
Pediatric ward	16 (9.52)	11 (12.36)
Perinatology ward	11 (6.55)	6 (6.74)
Clinic	7 (4.17)	5 (5.62)
Obstetrics and Gynecology ward	1 (0.60)	0 (0)

TABLE 2. Distribution of specimen types [n (%)] for isolating *K. pneumoniae* at Dr Soeradji Tirtonegoro General Hospital Klaten during June 2017-May 2018

Types of specimen	ESBL-producing <i>K. pneumoniae</i> isolates (n=89)	<i>K. pneumoniae</i> isolates (n=168)	Proportion ESBL-producing <i>K. pneumoniae</i> (%)
Sputum	36 (40.45)	76 (45.24)	36/76 (47.36)
Pus	12 (13.48)	27 (16.07)	12/27 (44.44)
Blood	18 (20.22)	20 (11.90)	18/20 (90)
Urine	8 (8.99)	12 (7.14)	8/12 (66.67)
Bronchial washings	1 (1.12)	9 (5.36)	1/9 (11.11)
Feces	5 (5.62)	6 (3.57)	5/6 (83.33)
Wound swab	3 (3.37)	5 (2.98)	3/5 (60)
Sterile site	2 (2.25)	7 (4.17)	2/7 (28.57)
Others*	4 (4.49)	6 (3.57)	4/6 (66.67)

Out of 168 isolates of *K. pneumoniae*, 104 isolates were possible ESBL producers (61.90%), with 85.58% (89/104) confirmed as ESBL producers using the MDDST method (FIGURE 1). The proportion of *K. pneumoniae* confirmed

as ESBL producers was 52.98% (89/168) of the total isolates of *K. pneumoniae*. ESBL-producing *K. pneumoniae* were mostly obtained from patients treated in the ICU (41.57%) (TABLE 1).

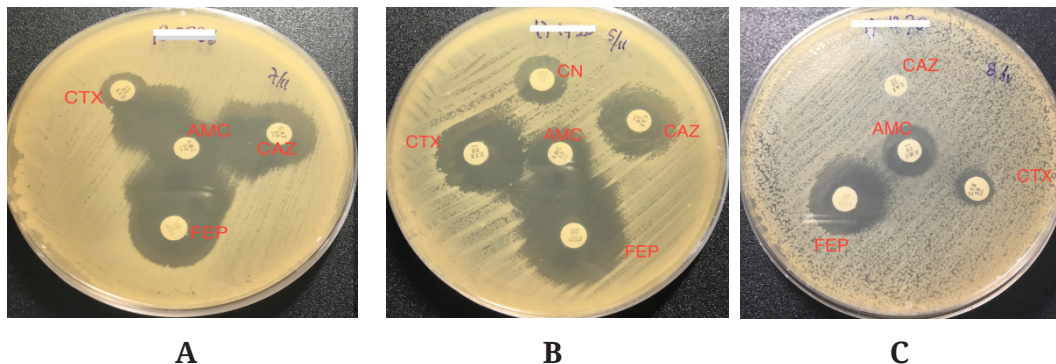


FIGURE 1. A positive MDDST for cephalosporin discs; A) A positive MDDST with an increased inhibition zone for ceftazidime, ceftaxime and cefepime towards amoxicillin clavulanate; B) A positive MDDST with increased inhibition zone for ceftaxime and cefepime; C) A positive MDDST with increased inhibition zone only for cefepime. AMC=amoxicillin clavulanate; CAZ=ceftazidime; CTX=ceftaxime; FEP=cefepime; CN=gentamicin

Based on the distribution of specimen types, ESBL-producing *K. pneumoniae* was mostly isolated from sputum (40.45%) (TABLE 2). Whereas if based on the proportion of ESBL producers, these isolates were often found in all specimens except those originating from bronchial washings. The proportion of ESBL-producing *K. pneumoniae* in sputum, pus, blood, and urine was 47.36%, 44.44%, 90% and 66.67%, respectively (TABLE 2).

TABLE 3 shows the percentage susceptibility of ESBL-producing *K.*

*pneumoniae* clinical isolates to various origin specimens using antibiotic panels according to the Indonesian Society for Clinical Microbiologist. In this study, 38 isolates of *K. pneumoniae* were derived from respiratory tract specimens, namely sputum, bronchial washings, and tracheal aspiration. ESBL-producing *K. pneumoniae* from respiratory tract specimens had a sensitivity of more than 80% to piperacillin-tazobactam (88.89%), amikacin (97.37%), and meropenem (100%). Sensitivity to cefepime and ceftriaxone were 0% in each.

TABLE 3. Percentage of susceptibility (%) of ESBL-producing *K. pneumoniae* isolates to antibiotics based on the origin of specimens at Dr. Soeradji Tirtonegoro General Hospital Klaten during June 2017-May 2018

Antibiotics	The origin of the specimens			
	Respiratory tracts (n=38)	Blood (n=18)	Wound (n=17)	Urine (n=8)
Cephazolin	0	-	11.76	0
Gentamycin	34.21	11.11	64.71	37.5
Tobramycin <sup>#</sup>	26.32	16.67	35.29	37.5
Amikacin <sup>#</sup>	97.37	100	82.35	87.50
Amoxicillin- Clavulanate	28.95	-	58.82	12.50
Piperacillin- Tazobactam <sup># *</sup>	88.89	75	75	100
Cefuroxime	2.63	0	5.88	0
Cefepime <sup>#</sup>	0	0	0	0
Ceftriaxone	0	0	5.88	0
Ciprofloxacin	-	66.67	17.65	62.50
Levofloxacin <sup>#</sup>	71.05	83.33	29.41	75
Meropenem <sup>#</sup>	100	100	100	100
Trimethoprim- Sulfamethoxazole	18.42	-	11.76	25
Nitrofurantoin	-	-	-	75

\*Not all ESBL-producing *K. pneumoniae* isolates were tested with piperacillin-tazobactam discs. The number of ESBL-producing *K. pneumoniae* isolates tested with piperacillin-tazobactam in the respiratory tract = 9, blood = 4, wounds = 4, urine = 1; #Antibiotics with restricted use based on Indonesian Society for Clinical Microbiologist.

## DISCUSSION

In this study, the proportion of *K. pneumoniae* isolates was 17.46% of total clinical bacterial isolates during June 2017-May 2018. This finding should be a major concern because *K. pneumoniae* is often associated with high levels of antibiotic resistance, nosocomial infections, and outbreaks in hospital.<sup>6</sup>

The ESBL-producing *K. pneumoniae* was mostly isolated from sputum. This is in line since *K. pneumoniae* often causes pneumonia, septicemia, UTI and wound infections.<sup>22</sup> The same results were also reported from Dr. Arifin Achmad General

Hospital Pekanbaru,<sup>16</sup> whereas research at Bugando Medical Center Tanzania reported that the pathogen originated mostly from urine.<sup>12</sup>

The majority of infections caused by *K. pneumoniae* are associated with hospitalization, especially in ICUs.<sup>6</sup> In this study, ESBL-producing *K. pneumoniae* was mostly isolated from patients treated in the ICU. Similar results were also reported at Dr. Arifin Achmad General Hospital Pekanbaru,<sup>16</sup> and Imam Reza Hospital of Mashhad Iran.<sup>11</sup> ESBL-producing *K. pneumoniae* are mainly obtained from ICUs for adults, neonates and premature infants.<sup>12</sup>

Accordingly, ICU is one of the risk factors of ESBL infection, because patients in intensive care unit are often given long-term broad-spectrum antibiotics, invasive procedures, and long-term catheter placements. Other risk factors are pressure sores, poor nutritional status, and travel history to Asia, Greece, Turkey, and the United States.<sup>11,23-26</sup>

In this study, the ESBL confirmation test was done with MDDST, which is a modification of the Double Disc Synergy Test (DDST) to increase sensitivity to bacteria that co-produce AmpC and ESBL enzymes. Many of *K. pneumoniae* isolates are able to produce both of those enzymes.<sup>27</sup> AmpC  $\beta$  lactamase hydrolyzes penicillins, cephalosporins, cephamycins, and monobactams but does not inhibit clavulanate.<sup>28</sup> Clavulanate induces high levels of AmpC expression in bacteria with AmpC and ESBL co-production, thereby hydrolyzing third generation cephalosporins. AmpC expression covers the synergy of third generation cephalosporins with amoxicillin-clavulanate.<sup>23</sup>

High level AmpC expression has minimal effect on cefepime, so that an increase in the inhibition zone between amoxicillin-clavulanate and cefepime discs can still be observed. If the synergy effect is only positive for cefepime but has a negative effect on third generation cephalosporins, isolates are suspected of co-production of AmpC and ESBL (20). MDDST using ceftazidime 30  $\mu$ g, cefotaxime 30  $\mu$ g, and cefepime 30  $\mu$ g, has a sensitivity of 100% and specificity of 93%.<sup>19</sup>

After confirmation testing, most of the *K. pneumoniae* isolates were identified as ESBL producers with ceftazidime, cefotaxime, and cefepime discs. Only a small number of isolates were confirmed as ESBL producers with cefotaxime or cefepime discs, because the optimal substrate profile varied among the ESBL enzymes.<sup>29</sup>

In this study, the proportion of

ESBL-producing *K. pneumoniae* isolates was 52.98%. The proportion of ESBL-producing bacteria is important to know because it correlates with an increase in antibiotic resistance. This causes limited choice of antibiotics. Sensitivity patterns of ESBL producing bacteria can help clinicians determine the right antibiotic for therapy. Similar results were reported from Dr. Soetomo General Hospital, Surabaya in 2011 (50.28%) and in 2012 (58%).<sup>14</sup> While higher yields were reported from Dr. Arifin Achmad General Hospital, Pekanbaru in 2015 (66.2%).<sup>16</sup> The difference in this study findings might be due to differences in ESBL detection methods, sampling techniques, study population, patient characteristics, guidelines for antibiotic use and infection control strategies.<sup>11,13,15</sup>

The number of ESBL producing bacteria is one indicator for the observation of outbreaks of MDR bacteria.<sup>30</sup> The high number of ESBL-producing *K. pneumoniae* among various hospitals in Indonesia, shows the large role of ESBL bacteria in antibiotic resistance. Evaluation of antibiotic usage and surveillance of MDR bacteria must be routinely done and reported periodically by the local Antimicrobial Stewardship Team in the relevant hospital.<sup>30</sup> Regulations are needed related to compliance with prudent antibiotic usage, especially cephalosporins.<sup>31</sup> In addition, compliance with the principle of standard precautions must be increased to prevent the spread of resistant bacteria through plasmids.<sup>32</sup>

Controlling the spread of ESBL-producing bacteria is done by identifying and immediately isolating patients with suspected ESBL-producing bacteria colonization or infection, conducting molecular epidemiological analyzes on strains of colonized or infected patients,<sup>23</sup> and also minimizing the length of treatment to reduce the likelihood of patients being a source of cross infection.<sup>33</sup> The spread of pathogens

and the risk of cross infection can be minimized by placing infected patients in isolation rooms. If the isolation room is not available, the patient should be placed in a cohorting way, which involves treating several patients with the same pattern of infection in one room.<sup>30</sup>

Precautions should always be applied since the proportion of ESBL-producing *K. pneumoniae* is high in sputum, pus, blood and urine. The principle of standard precautions must be done especially in hospitalized patients to avoid nosocomial infections. Lower respiratory tract infections should be of particular concern, especially in patients using endotracheal tubes.<sup>31</sup> Likewise, the use of an intravenous catheter, arterial catheter and urinary catheter should also be a concern for septicemia and urinary tract infection.

The results of bacterial sensitivity testing for antibiotics can be arranged into an antibiogram for empirical therapy guidelines. Antibiogram contains isolate data according to the type of specimen and room location.<sup>30</sup> The number of isolates needed to make the ideal antibiogram is at least 30.<sup>34</sup> In this study, the data that can be used was ESBL-producing *K. pneumoniae* isolates originating from the respiratory tract. Antibiotics that have a sensitivity of more than 80% can be used as empirical therapy.<sup>30</sup>

ESBL-producing *K. pneumoniae* isolates originating from the respiratory tract have a sensitivity of more than 80% against amikacin, piperacillin tazobactam, and meropenem. Although piperacillin-tazobactam has a sensitivity of more than 80%, not all isolates of *K. pneumoniae* originating from the respiratory tract were tested for sensitivity to piperacillin-tazobactam. So, it needs special consideration for piperacillin-tazobactam to be used as empirical therapy.

ESBL-producing *K. pneumoniae* isolates originating from other specimens

are less than 30 isolates each. Therefore, the results of sensitivity must be interpreted with special consideration. The sensitivity data of less than 30 isolates may cause an unreliable and unrepresentative antibiogram.<sup>34</sup>

The sensitivity of ESBL-producing *K. pneumoniae* with more than 80% were obtained for ertapenem, meropenem, and amikacin at Dr. Arifin Achmad General Hospital, Pekanbaru in 2015.<sup>16</sup> Whereas amikacin, cefoperazone-sulbactam, meropenem and phosphomycin were antibiotics which are reported to have a sensitivity of more than 80% in Dr. Soetomo Hospital Surabaya, Dr. Saiful Anwar General Hospital, Malang, and Dr. Kariadi General Hospital, Semarang in 2010.<sup>31</sup> This means an antibiotic choice with good sensitivity, is limited to antibiotics which are restricted such as meropenem, piperacillin-tazobactam, and amikacin.

Carbapenem is the treatment of choice for severe infections caused by ESBL-producing *K. pneumoniae*.<sup>35</sup> However, the increasing incidence of infection with ESBL-producing bacteria throughout the world, has led to an increase in the use of carbapenem. The use of excessive carbapenem triggers selection pressure resulting in carbapenem resistant bacteria.<sup>2</sup> Carbapenem resistance causes antibiotic resistance problems which are more difficult to overcome.

This study has limitations due to the scarcity of piperacillin-tazobactam discs. Not all *K. pneumoniae* isolates were tested for antibiotic susceptibility with piperacillin-tazobactam so that representative sensitivity data were not available.

## CONCLUSION

Proportion of ESBL-producing *K. pneumoniae* clinical isolates is 52.98% of total *K. pneumoniae* isolates at Dr. Soeradji Tirtonegoro General Hospital, Klaten.



Piperacillin-tazobactam, amikacin, and meropenem have a sensitivity of more than 80% in the respiratory tract sample isolates from patients at Dr. Soeradji Tirtonegoro General Hospital, Klaten.

## ACKNOWLEDGEMENTS

This study was financially supported by the *Dana Masyarakat Grant* (2017), Faculty of Medicine, Public Health, and Nursing, Universitas Gadjah Mada, Yogyakarta. The authors want to thank: dr. Hesty Lusinta Sp MK; dr. Delima Fajar Liana; dr. Luthvia Annisa; dr. Stefanie Juergen, MPH; Margono; Erna Yulis Setiawati; Retno Wulandari; and Mulyani for their technical assistance. We also want to thank Mr. Erik Christopher for critical editing of the manuscript.

## REFERENCES

- Ahmad N, Drew W, Lagunoff M, Pottinger P, Reller L, Sterling C. Sherris medical microbiology. Ryan J, Ray C, editors, 6th Ed. New York: Mc Graw-Hill, 2014.
- Woldu MA. *Klebsiella pneumoniae* and its growing concern in healthcare settings. *Clin Exp Pharmacol* 2016; 6(1):1-7.
- Tsai S, Huang J, Chen S, Hsu BR, Lin J, Huang S. Characteristics of *Klebsiella pneumoniae* bacteremia in community acquired and nosocomial Infections in diabetic patients. *Chang Gung Med J* 2010; 33(5):532-9.
- Harvey RA, Cornelissen CN, Fisher BD. Gastrointestinal gram negative rods. In: Lippincott's Illustrated Reviews Microbiology, 3<sup>rd</sup> Ed., Philadelphia. Lippincott: Williams& Wilkins, 2013; 617.
- Tille PM. Gram-negative bacilli and coccobacilli (macconkey-Positive, Oxidase-Negative) In: Bailey & Scott's Diagnostic Microbiology, 13th Ed. St Louis: Elsevier Mosby, 2014; 312.
- De Jesus MB, Ehlers MM, Santos RF Dos, Kock MM. World' s largest Science, technology & medicine open access book publisher review-understanding  $\beta$  lactamase producing *Klebsiella pneumoniae*. *Intech Open* 2015; 51-69. <https://doi.org/10.5772/61852>
- Perez F, Endimiani A, Hujer KM, Bonomo RA. The continuing challenge of ESBLs. *Curr Opin Pharmacol* 2007; 7(5):459-69. <https://doi.org/10.1016/j.coph.2007.08.003>
- Paterson DL. Resistance in gram-negative bacteria: *Enterobacteriaceae*. *Am J Infect Control* 2006; 34(5 SUPPL): 20-8. <https://doi.org/10.1016/j.ajic.2006.05.238>
- Zhang X, Reinert RR, Low DE, Wattal C, Dowzicky MJ. Antimicrobial susceptibility among organisms from the Asia/Pacific Rim, Europe and Latin and North America collected as part of TEST and the in vitro activity of tigecycline. *J Antimicrob Chemother* 2007; 60(9):1018-29. <https://doi.org/10.1093/jac/dkm310>
- Zhang J, Zhou K, Zheng B, Zhao L, Shen P, Ji J. High prevalence of ESBL-producing *Klebsiella pneumoniae* causing community-onset Infections in collection of clinical Isolates. *Front Microbiol* 2016; 7(11): 1-10. <https://doi.org/10.3389/fmicb.2016.01830>
- Ashrafian F, Askari E, Kalamatizade E, Javad M, Shahroodi G, Naderi-Nasab M. The frequency of Extended Spectrum Beta Lactamase (ESBL) in *Escherichia coli* and *Klebsiella pneumoniae*: a report from Mashhad, Iran. *J Med Bacteriol* 2013; 2(1-2):12-19.
- Mshana SE, Kamugisha E, Mirambo M, Chakraborty T, Lyamuya EF. Prevalence of multiresistant gram-negative organisms in a tertiary hospital in Mwanza, Tanzania. *BMC Res Notes* 2009; 2:1-6. <https://doi.org/10.1186/1756-0500-2-49>
- Kuntaman, Mertaniasih NM, Hadi

- U. Multiresistance pattern of Extended Spectrum Beta Lactamase (ESBL) *Escherichia coli* and *Klebsiella pneumoniae* strains. Folia Med Indonesiana 2006; 42(1):40-46.
14. Hadi U, Qibtiyah M, Paraton H. Problem of antibiotic use and antimicrobial resistance in Indonesia: are we really making progress? Indones J Trop Infect Dis 2013; 4(4):5-8.  
<https://doi.org/10.20473/ijtid.v4i4.222>
  15. Fitri NN, Rusli M, Wahyunitisari MR. Antibiotic use is not a risk factor of infections by Extended Spectrum Beta Lactamase producing bacteria in Dr Soetomo Hospital Surabaya. Microbiol Indones 2015; 9(4):150-6.  
<https://doi.org/10.5454/mi.9.4.2>
  16. Anggraini D, S UH, Savira M, D FA, Irawan D, R RP. Prevalensi dan pola sensitivitas *Enterobacteriaceae* penghasil ESBL di RSUD Arifin Achmad Pekanbaru. J Kedokt Brawijaya 2018; 30(1):47-52.  
<https://doi.org/10.21776/ub.jkb.2018.030.01.9>
  17. Warganegara E, Apriliana E. The determining type of Extended-Spectrum  $\beta$  Lactamase Enzyme (ESBL) from *Escherichia coli* resistance cephalosporine of third generation in RSUD Abdoel Moeloek Bandar Lampung. J Kedokt Fak Kedokt Univ Lampung 2014; 4(7):87-96.
  18. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI document M100. 28th Ed. Wayne, PA: Clinical and Laboratory Standards Institute, 2018. 224.
  19. Garrec H, Drieux-Rouzet L, Golmard JL, Jarlier V, Robert J. Comparison of nine phenotypic methods for detection of Extended-Spectrum  $\beta$ -Lactamase production by *Enterobacteriaceae*. J Clin Microbiol 2011; 49(3):1048-57.  
<https://doi.org/10.1128/JCM.02130-10>
  20. Kaur J, Chopra S, Sheevani, Mahajan G. Modified double disc synergy test to detect ESBL production in urinary isolates of *Escherichia coli* and *Klebsiella pneumoniae*. J Clin Diagnostic Res 2013; 7(2):229-33.  
<https://doi.org/10.7860/JCDR/2013/4619.2734>
  21. Mahon C, Lehman DC, Manuselis G. Laboratory Identification of Significant Isolates. In: Wurm-Cutter E, editor. Textbook of Diagnostic Microbiology. 5th Ed. Maryland Heights: Saunders Elsevier, 2015. 428-9.
  22. Podschun R, Ullmann U. *Klebsiella* spp. as nosocomial pathogens: epidemiology, taxonomy, typing methods, and pathogenicity factors. Clin Microbiol Rev 1998; 11(4):589-603.  
<https://doi.org/10.1128/CMR.11.4.589>
  23. Paterson DL, Bonomo RA. Extended Spectrum  $\beta$  Lactamases: a clinical update. Clin Microbiol Rev 2005; 18(4):657-86.  
<https://doi.org/10.1128/CMR.18.4.657-686.2005>
  24. Lautenbach E, Patel JB, Bilker WB, Edelstein PH, Fishman NO. Extended Spectrum  $\beta$  Lactamase producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin Infect Dis 2001; 32(3): 1162-71.  
<https://doi.org/10.1086/319757>
  25. Broberg CA, Palacios M, Miller VL. *Klebsiella* : a long way to go towards understanding this enigmatic jet-setter. F1000 prime Reports 2014; 12(8):1-12.  
<https://doi.org/10.12703/P6-64>
  26. Ghafourian S, Sadeghifard N, Soheili S, Sekawi Z. Extended Spectrum  $\beta$  Lactamases : definition, classification and epidemiology. Curr Issues Mol Biol 2015; 17:11-22.
  27. Diab AM, Abul-Aziz MH, El-Kholy IMA, Rezk MA. Modified Double Disc Synergy Test (MDDST) versus Double Disc Synergy Test (DDST) for detection of ESBL(S) in Ampc

- B-Lactamase producing *Klebsiella* clinical isolates. Eur Chem Bull 2018; 7(2): 89.  
<https://doi.org/10.17628/ecb.2018.7.89-92>
28. Black JA, Moland ES, Thomson KS. AmpC disc test for detection of plasmid mediated AmpC  $\beta$  Lactamases in Enterobacteriaceae lacking chromosomal AmpC  $\beta$  Lactamases. J Clin Microbiol 2005; 43(7):3110-3.  
<https://doi.org/10.1128/JCM.43.7.3110-3113.2005>
  29. Rawat D, Nair D. Extended Spectrum  $\beta$  Lactamases in gram negative bacteria. J Global Infec Dis 2010; 2(3):263.  
<https://doi.org/10.4103/0974-777X.68531>
  30. Kementerian Kesehatan Republik Indonesia. Peraturan Menteri Kesehatan Republik Indonesia Nomor 8 tahun 2015 tentang Program Pengendalian Resistensi Antimiroba di Rumah Sakit. Jakarta: 2015.
  31. Kuntaman K, Santoso S, Wahjono H, Mertaniasih NM, Lestari ES, Farida H. The Sensitivity Pattern of Extended Spectrum  $\beta$  Lactamase-Producing bacteria against six antibiotics that routinely used in clinical setting. J Indones Med Assoc 2011; 61(12):482-6.
  32. Kementerian Kesehatan Republik Indonesia. Peraturan Menteri Kesehatan Republik Indonesia Nomor 240 tahun 2011 tentang Pedoman Umum Penggunaan Antibiotik. Jakarta: 2011.
  33. Warren RE, Harvey G, Carr R, Ward D, Doroshenko A. Control of infections due to Extended Spectrum  $\beta$  lactamase producing organisms in hospitals and the community. Clin Microbiol Infect 2008; 14(SUPPL. 1):124-33.  
<https://doi.org/10.1111/j.1469-0691.2007.01870.x>
  34. Clinical and Laboratory Standards Institute. Analysis and presentation of cumulative antimicrobial susceptibility test data. CLSI document M39A-A2, 2nd Ed. Reston, VA: Clinical and Laboratory Standards Institute; 2005.
  35. Harris PNA, Yin M, Jureen R, Chew J, Ali J, Paynter S. Comparable outcomes for  $\beta$  lactam/ $\beta$  lactamase inhibitor combinations and carbapenems in definitive treatment of bloodstream infections caused by cefotaxime-resistant *Escherichia coli* or *Klebsiella pneumoniae*. Antimicrob Resist Infect Control 2015; 4(14):1-10.  
<https://doi.org/10.1186/s13756-015-0055-6>