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Comparison of Sensitivity Enterobacteriaceae of Extended Spectrum Beta-Lactamase (ESBL) against Antibiotics of Quinolone and Carbapenem Group in Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia

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ABSTRACT

Antibiotic resistance is a challenge in medical problems. One prevalence of resistance that tends to expand globally is against ESBL-producing Enterobacteriaceae, a group of bacteria capable of destroying beta-lactam antibiotics. The known ESBL producing bacteria are *E. coli* and *K. pneumoniae*. This study aims to compare the sensitivity of quinolone and carbapenem antibiotics to ESBL-producing bacteria based on data obtained from Clinical Microbiology Laboratory, Faculty of Medicine, Universitas Indonesia through 2018-2019. Using the Vitek 2® Compact identification method, the results showed that the prevalence of *E. coli* and *K. pneumoniae* ESBL was positive less than 5%. All of the ESBL–producing *E. coli* came from urine specimens, while ESBL-producing *K. pneumoniae* came from different types of specimens which are sputum and blood. Most prevalence comes in the age range >50 years with female gender. In general, antibiotic sensitivity to the quinolones was less than 50% against ESBL-producing *E. coli*. Meanwhile, the sensitivity of carbapenem antibiotics reached 100% both against ESBL-producing *E.coli* and *K.pneumoniae*.

Keywords : ESBL, *E.coli, K. pneumoniae*, antibiotic sensitivity, quinolone, carbapenem

INTRODUCTION

Extended Spectrum Beta Lactamase (ESBL) is often interpreted as an enzyme produces by a microorganism that can provide resistance to other beta-lactam antibiotics, including penicillin, cephalosporin, aztreonam (except cephamycin and carbapenem) and also found in other resistance combinations thus causing multiresistant. This resistance is caused by bacteria by hydrolyzing the antibiotic [1].

The most common group of *Enterobacteriaceae* that has resistance to beta-lactam antibiotics is *Escherichia coli (E. coli)* and *Klebsiella pneumoniae* (*K. pneumoniae*). These bacteria work with 3 mechanisms, namely destroying the antibiotic class using enzyme beta-lactamase, the ability of antibiotic penetration in binding to the protein trans peptidase and decreasing the affinity between the beta-lactam class of antibiotics with certain protein [2,3].

Currently various countries have reported this incidence with varying prevalence. The regions that become one of the centre of the ESBL global pandemic region are the Middle

Easter with the prevalence of ESBL-producing *Enterobacteriaceae* reaching up to 61%. In India the ESBL infection ranges from 13,5%-73,5% for *E. coli* and 17%-73,5% for *K. pneumoniae.* Meanwhile, based on data obtained from various national referral hospitals in Indonesia, the prevalence of ESBL reaches 26,7%-56,8% for *E. coli* and 32,1%-56,8% for *K. pneumonia* [4,5].

Antibiotic resistance is the ability of microorganism to fight one or more microbial agents. The consequences that occur can be very severe and varied. The development of antibiotic resistance is a naturally occurring phenomenon caused by gene mutations or the acquisition of exogenous resistance genes that carry genetic material and spread horizontally between bacteria. Bacteria are able to get multiple resistance, which results in resistance to some antibiotics. If this happens, it can lead to limited antibiotic therapy as a treatment.

Antibiotic resistance is one of the challenges in the word of medicine. Patients who have been resistant to antibiotics during their treatment, have a worse prognosis and require more intensive [6,7]. Due to the high rate of antibiotic resistance, this study aims to examine the comparison of ESBL sensitivity to antibiotics from carbapenem and quinolones from patients with various clinical specimens because until now empirical therapy for ESBL infection uses both antibiotics.

METHODS

Specimen collection

Specimens taken according to the type of specimens are then accommodated in sterile pots with screw cap, not easily broken and not leaking. Pots are provided by the laboratory. Specimens which are not immediately on process are stored in the 4^{o}C. Specimens derived from various clinical samples sent to microbiology laboratories FKUI for the period 2018-2019.

Preparation of slide

Each specimen was stained with Gram (Becton Dickinson) according to standard procedures.

Culture

Specimens were inoculated onto Blood Agar, McConkey Agar and Chocolate Agar (Merck), incubated in an incubator (Thermo), 35^{o}C for 24 hours.

Identification

Gram staining is carried out according to the procedure of each colony that grows on the medium. Followed by identification using the automatic machine Vitek 2® Compact (Biomerieux). Identification and resistance examination were performed using the Vitek GN tools. This tools uses the principle of automatic biochemistry which takes about 18 hours for process. There are 3 stages in the examination, namely preparation and standardization turbidity of the inoculums, entering data with barcode system and inserting the card into the instrument. Furthermore, the entire process of inoculation, incubation, reading, validation and interpretation of results are done automatically by the tool.

Isolates

Number of isolates was 282, each consisting of 155 for *E. coli* and 127 for *K. pneumoniae.*

RESULTS AND DISCUSSION

Culture results showed that 5 (3,22%) of 155 *E. coli* isolates were detected as ESBL. While only 2 isolates (1,57%) indicated positive ESBL *K. pneumoniae* from 127 isolates. In total there were 7 isolates (2,48%) positive from 282 isolates that were identified (Table 1). This study indicates that *E. coli* producing ESBL has a higher percentage than *Klebsiella* producing ESBL. This result is lower compared with a study conducted by Anggraini D, *el al* in Arifin Achmad Pekanbaru Hospital in 2017. Their study showed *E.coli* producing ESBL was 62,2% while *K. pneumoniae* was 66,2 % [8]. Although the results of this study are different, one thing indicates that *E. coli* and *Klebsiella* pneumonia are the most common bacteria that cause ESBL.

Based on the type of specimen, in 155 patients infected *E. coli* there were 13 different types of specimens from a total of 19 specimens.

Meanwhile, in 127 patients infected *K. pneumoniae* there were 15 different types of specimens from a total 19 types (Table 2). ESBL-producing *E. coli* came entirely from the same type of specimen that is urine, while ESBL-producing *K. pneumoniae* found 2 different types of specimens, such as sputum and blood (Table 3). It seems that this related to *E. co*li as the most common etiology of urinary tract infections [8].

Table 1. Prevalence of ESBL-producing *E. coli* and *K. pneumoniae* in 2018-2019

Bacteria	*E. coli* (n=155)		*K. pneumoniae* (n=127)		Total (n=282)	
	n	%	n	%	n	%
ESBL	5	3,22	2	1,57	7	2,48
Non ESBL	150	96,7	125	98,4	277	98,2

Table 2. Prevalence *E. coli* and *K. pneumoniae* by type of specimen

Type of specimen	*E.coli*	*K. pneumoniae*
Urine	90	31
Pus	22	12
Tissue	6	6
Blood	5	7
Fluid	1	0
Sputum	6	39
Cervix	4	2
The wound	4	1
Abscess	1	4
Vagina	3	1
Throat	0	2
Alveolar-bronchi	1	8
Pleural fluid	0	1
Bone	0	1
Breast milk	0	9
Tracheal aspirate	0	1
Urethra	1	0
Total	155	127

Table 3. Prevalence of ESBL-producing *E. coli* and *K. pneumoniae* based on the type of specimen

Specimen Type	*E. coli*		*K. pneumoniae*	
	ESBL-Producing	Prevalence (%)	ESBL-Producing	Prevalence (%)
Urine	5	3,22	0	0
Sputum	0	0	1	0,78%
Blood	0	0	1	0,78%
Total	5	3,22%	2	1,57%

Distribution of patients with *E. coli* and *K. pneumoniae* based on gender and age showed varied results as shown in Table 4. On the *E.coli* infection, female were more numerous than male (77 from 155), whereas infection by *K. pneumoniae*, male more numerous than female (60 from 127). However, unfortunately there are about 33 were infection by *E. coli* and 16 by *K. pneumoniae* whose gender is not known because there is no data. *E. coli* percentage the highest in women while *K. pneumoniae* in men, when we observed at the type of specimen, it is found that urine specimens are the most identified. Thus seems to berelated to bacterial causes, where it is known that the most common cause of urinary tract infection (urine specimen) is over 90% *of E. coli* as etiology. The highest age range in *E.coli* and *K. pneumoniae* was founded ages over 50 years, respectively 32,25% and 50,39% (Table 4).

Comparison of age and gender variable distribution in ESBL-producing of *E.coli* and *K. pneumoniae* is shown in Table 5. ESBL-producing *E.coli* all patients were female (100%) whereas in *K. pneumoniae* were male and female respectively 50%. The age range found in *E.coli* is above 50 years while *K. pneumoniae* at age 31-50 and above 50 years respectively 50% . Elderly is one of the risk factors for ESBL infection. This is partly due to a decrease in the immune system [9,10,11]. This study is an accordance with Tham J *et al* (2013) which shows that the demographic characteristics of the subjects studied have an average 65 years with the most female [12,13,14].

Table 4. Distribution of patients with *E. coli* and *K. pneumoniae* based on gender and age

Variable	*E. coli*	*K. pneumoniae*
Gender		
Male	45	60
Female	77	51
Not known	33	16
Age (years)		
<1	0	0
1-15	33	18
16-30	10	6
31-50	31	22
>50	50	64
Not known	31	17
Total	155	127

The sensitivity of ESBL-producing of *E.coli* and *K. pneumoniae* to quinolone and carbapenem is shown in Table 6. The sensitivity of ciprofloxacin to ESBL-producing *E.coli* is 41,5% while that *K. pneumonia* is 100% resistant. Meanwhile, the antibiotic sensitivity of carbapenem groups to *E.coli* and *K. pneumoniae* producing ESBL, respectively reached 100%.

Table 5. Distribution of ESBL-producing *E. coli* and *K. pneumoniae* based on gender and age

Variable	ESBL-producing	
	E. coli	*K. pneumoniae*
Gender		
Male	0	1
Female	5	1
Age (years)		
<1	0	0
1-15	0	0
16-30	0	0
31-50	0	1
>50	5	1
Total	5	2

Table 6. Sensitivity ESBL-producing *E. coli* and *K. pneumoniae* against Quinolone and Carbapenem

Antibiotics	*E.coli*	*K. pneumoniae*
Quinolone		
Ciprofloxacin	41,5%	0%
Carbapenem		
Meropenem	100%	100%
Ertapenem	100%	100%

ESBL enzyme-producing bacteria, such as *E. coli* and *K. pneumoniae* are generally associated with quinolone group resistance and some with carbapenem. This happens because of the mechanism of resistance of microorganisms of this antibiotics. The regulation of the resistance mechanism that occurs is located in the plasmid gene, which is similar to the process of ESBL enzyme formation [15,16,17]. This theory supports the results of this study, which outline the sensitivity of quinolone antibiotics to *E. coli* and *K. pneumoniea* that produce ESBL under 50%. Carbapenem is an effective antibiotic for ESBL because it is resistant to β-lactam hydrolysis, but the use of carbapenem freely and freely has the risk of becaming resistant because it can be hydrolyzed by the metallo β-lactamase enzyme [18].

CONCLUSION

The prevalence of ESBL-producing *E. coli* and *K. pneumoniae* is less than 5%. ESBL-producing *E. coli* is entirely derived from urine specimens while *K. pneumoniae* is derived from sputum and blood. Patients with an age range less than 50 years with female occupy the highest prevalence. The sensitivity of the quinolone (ciprofloxacin) of antibiotics to ESBL-producing *E. coli* was 41,5% whereas in *K. pneumoniae* were completely resistant. Meanwhile, the antibiotic sensitivity of carbapenem groups reaches 100% both of *E. coli* and *K. pneumoniae* that produce ESBL. Carbapenem can be the antibiotic of choice for management of infections by the ESBL bacteria.

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