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Antibiotic Use Is Not a Risk Factor of Infection by Extended-Spectrum Beta-Lactamase Producing Bacteria in Dr. Soetomo Hospital Surabaya

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Infection by *Extended-Spectrum Beta-Lactamase* (ESBL) producing bacteria confers a major challenge for clinicians due to limited treatment options and poor prognosis. Inappropriate antibiotic use is thought to cause the emergence of this resistant strain through selective pressure mechanisms. This study aims to describe the proportion of ESBL-producing bacteria and characteristics of patients with ESBL-producing bacterial infection, and to analyze the risk factors of infection by ESBL-producing bacteria in Dr. Soetomo Hospital, Surabaya. A cross-sectional study was conducted on medical records of inpatients of Internal Medicine Ward of Dr. Soetomo Hospital. Samples were classified into ESBL-positive or ESBL-negative groups. Demographic data, clinical data and previous antibiotic use of 66 samples (33 in each group) were retrospectively obtained. As many as 30 patients (45.5%) were male. Mean age of patients in the ESBL-positive and negative group were 53.57 (±16.77) and 54.27 (±14.88) years, respectively ($p>0.05$). The median pre-infection length of stay was 4 and 3 days for ESBL-positive and negative group, respectively ($p>0.05$). Type 2 diabetes mellitus was the most common comorbid disease (33.3%). The most frequent bacteria obtained from clinical isolates was *Escherichia coli* (49.3%). Proportion of ESBL producers amongst *E.coli* and *K. pneumoniae* isolates were 75% and 38.5%, respectively. The most frequently prescribed empirical antibiotic was ceftriaxone. None of the antibiotic used were risk factors for infection by ESBL-producing bacteria. Although none of the assessed variables were risk factors for ESBL-infection was discovered, this study finds a significantly larger proportion of ESBL-*E. coli* compared to non-ESBL producing *E. coli*. Further studies should include larger sample size and quantitatively measured antibiotic use.

Key words: antibiotic resistance, antibiotic use, ESBL, *Escherichia coli*

Infeksi oleh bakteri penghasil *Extended-Spectrum Beta-Lactamase* (ESBL) menjadi sebuah tantangan bagi klinisi karena terbatasnya pilihan terapi antibiotic dan prognosis pasien yang buruk. Penggunaan antibiotik secara tidak tepat diduga menyebabkan berkembangnya melalui tekanan seleksi. Penelitian ini bertujuan untuk mendeskripsikan prevalensi bakteri penghasil ESBL dan karakteristik pasien yang terinfeksi oleh bakteri penghasil ESBL serta menganalisis faktor risiko infeksi oleh bakteri penghasil ESBL di RSUD Dr. Soetomo, Surabaya. Sebuah studi cross-sectional dilakukan menggunakan rakam medic pasien rawat inap di instalasi rawat inap medik penyakit dalam RSUD. Dr. Soetomo, Surabaya. Sampel dikelompokkan menjadi kelompok ESBL-positif dan ESBL-negatif. Data demografis, klinis dan riwayat penggunaan antibiotik dari 66 sampel (33 sampel pada masing-masing kelompok) dilihat secara retrospektif. Sebanyak 30 (45.5%) pasien berjenis kelamin laki-laki. Rata-rata usia pada kelompok ESBL-positif ialah 53.57(±16.77) tahun, sedangkan pada kelompok ESBL-negatif ialah54.27 (±14.88) tahun ($p>0.05$). Nilai median lama rawat inap sebelum infeksi pada kelompok ESBL-positif dan ESBL-negatif masing-masing 4 dan 3 hari ($p>0.05$). Penyakit komorbid tersering yang dijumpai pada pasien adalah diabetes mellitus (33.3%). Bakteri yang terbanyak didapatkan pada isolat klinis ialah *Escherichia coli* (49.3%). Prevalensi *E. coli* penghasil ESBL adalah 75% dan 38.5% pada *Klebsiella pneumoniae*. Antibiotik yang paling sering diberikan pada pasien ialah cephalosporin generasi-3 (ceftriaxon). Tidak didapatkan hubungan antara penggunaan antibiotik dengan infeksi oleh bakteri penghasil ESBL. Isolat *E. coli* yang menghasilkan ESBL secara signifikan lebih banyak daripada isolat yang tidak menghasilkan ESBL. Penelitian berikutnya diharapkan dapat melibatkan sampel yang lebih besar serta mengukur penggunaan antibiotik secara kuantitatif.

Kata kunci: ESBL, *Escherichia coli,* penggunaan antibiotik, resistensi antibiotik.

Extended-Spectrum Beta-Lactamase (ESBL) is a group of enzymes responsible for the resistance of gram negative bacteria towards beta-lactam antibiotics. These enzymes are mainly produced by members of the gram negative family Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae,* but other species such as *Acinetobacter baumanii* and *Pseudomonas aeruginosa*

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are also known to produce ESBLs (Dhillon and Clark 2012). ESBLs are able to hydrolize and inactivate beta-lactam antibiotics, including all penicillins, cephalosporin, and aztreonam, but has no activity against cephamycin and carbapenem (Tham 2012). This strain also commonly shows co-resistance towards fluoroquinolones and aminoglycosides, therefore limiting treatment options (Kaya *et al*. 2013). Limited susceptible antibiotic options have become the main challenge in treating infection by ESBL producing bacteria, causing an increase in medical costs, morbidity and mortality (Tumbarello *et al*. 2006).

The prevalence of ESBL producing bacteria varies among countries; however, an increasing trend is reported (Shaikh *et al*. 2015). The prevalence of ESBL-producing bacteria could be as low as 7.5% in North America to as high as 22% in the Asia-Pacific region (Dhillon and Clark 2012). Hadi *et al*. (2013) reported that the prevalence of ESBL-producing bacteria in institutions participating in the AMRIN (Antimicrobial Resistance in Indonesia: Prevalence and Prevention) Study increased from 22% in 2010 to 53% in 2012.

One of the notorious risk factors for infection by ESBL-producing bacteria is inappropriate antibiotic use. Antibiotic use is thought to drive selection pressure, eliminating susceptible bacteria and enabling the growth of resistant bacteria (Wright 2010). However, results obtained from previous studies vary and analytical studies on this topic are still limited in Indonesia. This study is conducted to describe the proportion of ESBL-producing bacteria; the clinical characteristics of patients with ESBL-producing bacterial infection, and to analyze the risk factors of infection by ESBL-producing bacteria in Dr. Soetomo Hospital, Surabaya. This study is by far the most recent analytical study on risk factors of infection by ESBL producing bacteria in a tertiary hospital in Indonesia.

MATERIALS AND METHODS

Study Design and Population. This cross sectional study involved medical records of patients with bacterial infection hospitalized at the Internal Medicine Ward of Dr. Soetomo Hospital, a tertiary referral hospital in Surabaya, Indonesia. The inclusion period was October 2014 to May 2015. The eligible population consists of patients with a diagnosis of bacterial infection from which clinical specimens have been collected for bacterial identification and antibiotic susceptibility testing at the Clinical Microbiology Laboratory of Dr. Soetomo Hospital. The minimum age of patients included in the study was 18 years old. Incomplete medical records were excluded. Samples were selected consecutively and were classified into two groups, ESBL-negative and ESBL-positive with a 1:1 ratio. Bacterial identification was conducted with the MicrobactTM System (Medvet diagnostics, Thebarton, Adelaide, Australia), ESBL-positive group consisted of patients with confirmed bacterial identification from clinical specimens, in which the identified bacteria was confirmed as ESBL-producing, while ESBL-negative group consisted of patients infected by a non-ESBL-producing bacteria. ESBL production by gram negative bacteria screening was done with disk diffusion testing using cefotaxime or ceftazidime based on reccomendations of the Clinical and Laboratory Standards Institute (CLSI) guidelines or by the BD Phoenix Automated Microbiology System (BD Diagnostic Systems, Sparks, MD) machine used in Clinical Microbiology Laboratory of Dr. Soetomo Hospital.

Data Collection. Medical records were reviewed to obtain necessary data. Studied variables were patient age and sex; comorbid disease and length of hospital stay (LOS) prior to infection; antibiotic use and the type of clinical specimen collected. Antibiotic use was defined as any class of antibiotic given to the patient before date of clinical specimen collection, with a minimum duration of antibiotic use of 48 h.

Statistical Analysis. Bivariate analysis on categorical variables was performed with chi square test or fisher's exact test when appropriate. Independent samples T-test was used to analyze association of age with infection by ESBL-producing bacteria. Independent samples Mann Whitney U Test was used to compare pre-infection length of hospital stay in the ESBL-positive and ESBL-negative group. All statistical analyses are significant at $p<0.05$; 95% Confidence Interval. All statistical analysis was conducted with SPSS version 20.

Ethical Clearance. The study protocol was approved by the Medical Research Ethics Committee of Dr. Soetomo Hospital, Surabaya, Indonesia [Komite Etik Penelitian Kesehatan Rsud Dr. Soetomo] (Ethical clearance approval number 135/Panke.KKE/II/2015).

RESULTS

Samples that met the inclusion criteria consisted of 66 medical records, 33 in each ESBL-positive and ESBL-negative group. As many as 30 patients (45.5%)

were male. Demographic and clinical characteristics of samples are shown (Table 1). There are no significant differences between the demographic characteristics of the ESBL-positive and ESBL-negative group (p>0.05). The most common comorbid diseases in samples were type 2 diabetes mellitus and malignancy. None of the comorbidities were found to be risk factors of infection by ESBL-producing bacteria.

From the 66 patients, 73 clinical specimens were positive for bacterial cultures. The majority of isolates were from urinary specimens (63.01%) Other clinical specimens obtained and ESBL production characteristics are shown (Table 2).

From 73 bacterial isolates, the most common species obtained was *Escherichia coli* (47.95%). From the 33 ESBL-positive isolates, as many as 27 (81.8%) isolates were *Escherichia coli*, five (15,2%) were *Klebsiella pneumonia* and one (3%) was *Enterobacter aerogenes* (Table 3). The number of ESBL-positive *E. coli* is significantly larger than the number of ESBL-negative isolates (p=0.000). The proportion of ESBL-producing isolates was 75% in *Escherichia coli* and 38.46% in *Klebsiella pneumoniae*.

Antibiotics used by patients were classified based its classes, including cephalosporin, beta-lactam/beta lactamase inhibitor, fluoroquinolones, and other types of antibiotics. Patients with combination of antibiotics is catagorized as those who receive more than one types of antibiotic simultaneously. The most frequently prescribed antibiotic is the third generation cephalosporin, ceftriaxone.

Bivariate analysis of antibiotic uses and infections by ESBL-producing bacteria shows that none of the antibiotics were risk factors for infection by ESBL-producing bacteria (Table 4).

DISCUSSION

Infection by *Extended-Spectrum Beta-Lactamase* producing bacteria has become a major challenge for clinicians due to increasing prevalence, limited antibiotic options, and poor prognosis. It is therefore

Table 1 Characteristics of samples

Characteristics	ESBL-positive N=33	ESBL-negative N=33	p
Demographic			
Gender (male)	12 (36.37%)	18 (54.54%)	0.138
Mean Age (±SD)	53.57 (16.77)	54.27 (14.88)	0.859***
Median pre-infection LOS (days)	4	3	0.547**
Comorbidities			
Type 2 Diametes Mellitus	4 (12%)	7 (21%)	0.322
Diabetic nephropathy	6 (18%)	4 (12%)	0.492
Malignancy	8 (24%)	9 (27%)	0.778
Renal disease	6 (18%)	4 (12%)	0.492
Liver disease	1 (3%)	2 (6%)	1.000*
Rheumatic diseases	2 (6%)	1 (3%)	1.000*

*p value generated with Fisher's exact test
** p value generated with Independent samples Mann Whitney U Test
*** p value generated with Independent samples T Test

Table 2 Sources of clinical specimens

ESBL	Clinical Specimens			
	Urine	Sputum	Pus	Blood
ESBL +	26 (56.5%)	5 (41.7%)	1 (10%)	1 (20%)
ESBL -	20 (43.5%)	7 (68.3%)	9 (90%)	4 (80%)
Total	46 (100%)	12 (100%)	10 (100%)	5 (100%)

Table 3 Distribution of bacteria in the ESBL-positive and ESBL-negative group

Bacteria	ESBL-positive	ESBL-negative	Total (%)
Escherichia coli	27	9	36 (49.3%)
Klebsiella pneumoniae	5	8	13 (17.8%)
Enterobacter aerogenes	1	2	3 (4.1%)
Enterobacter cloacae	0	1	1 (1.4%)
Proteus mirabilis	0	1	1 (1.4%)
Pseudomonas aeruginosa	0	6	6 (8.2%)
Acinetobacter baumanii	0	4	4 (5.5%)
Acinetobacter iwolfii	0	1	1 (1.4%)
Acinetobacter junii	0	1	1 (1.4%)
Cedecea lapagei	0	4	4 (5.5%)
Pasteurella mucocida	0	1	1 (1.4%)
Enterococcus faecalis	0	1	1 (1.4%)
Staphylococcus epidermidis	0	1	1 (1.4%)
Total	33	40	73 (100%)

Table 4 Analysis of antibiotic use in ESBL-positive and ESBL-negative group

Antibiotic	ESBL-positive	ESBL-negative	P	RR	95%CI (Lower-upper)
Cephalosporin	27	29	1.000	1.000	0.477-2.094
Ceftriaxone	25	25	1.000	1.000	0.570-1.756
Cefotaxime	1	0	1.000*		
Cefixime	0	1	1.000*		
Ceftazidime	3	3	1.000*	1.000	0.432-2.315
Beta Lactam-Beta Lactamase Inhibitor	0	2	0.492*		
Amoxicillin-Clavulanic Acid	0	1	1.000*		
Cefoperazone-Sulbactam	0	1	1.000*		
Fluoroquinolones	14	12	0.614	0.882	0.545-1.429
Ciprofloxacin	7	6	0.757	1.098	0.619-1.947
Levofloxacin	7	6	0.757	1.098	0.619-1.947
Other classes					
Meropenem	1	0	0.495*		
Azithromycin	0	1	1.000*		
Cotrimoxazole	1	1	1.000*	1.000	0.245-4.085
Combination of antibiotics	9	10	0.786		0.535-1.609

*p value generated with Fisher's exact test

*RR=relative risk

important to study the epidemiology and risk factors for acquisition of this resistant strain to provide adequate infection control in the hospital. A continuous surveillance should also be conducted because the prevalence usually changes overtime and differs in each health centers.

Demographic characteristics of patients obtained in this study showed that neither gender nor age were significant risk factors for infection by ESBL-producing bacteria. However, other studies with larger samples have found differing results. Older age is found to be a risk factor in a multinational survey on risk factors for infection by ESBL-producing bacteria (OR=2.4; 95%CI=1.6–3.6) (Ben-Ami *et al*. 2009). However, a contrasting result was found by Soraas *et al*., (2013) that younger age was found to be a risk factor for infection by ESBL-producing bacteria. Male sex was found to be a risk factor for infection by ESBL-producing bacteria in a multinational survey (Ben-Ami *et al*. 2009) (OR=2.5; 95%CI=1.7-3.7). Another study by Tuon *et al.* (2010) in Brazil also reported similar results (OR=2.62; 95%CI=1.16-5.93). However, Lee *et al*. (2010) reported that female sex was a risk factor for urinary tract infection by ESBL-producing bacteria (OR=1.44; 95%CI=1.062-1.951; p= 0.019).

Length of hospital stay previous to infection is also not a risk factor in this study. This is in concert with studies by Tuon *et al*., (2011) and Huang *et al*., (2007) which did not found that pre- infection length of hospital stay as risk factors for infection by ESBL-producing bacteria. However a case control study in an Australian tertiary hospital found that pre-infection length of hospital stay was a risk factor for colonization by ESBL producing bacteria (OR=1.2; 95%CI=1.0-1.3) (Osthoff *et al*. 2015).

None of the comorbidities were risk factors of infection by ESBL-producing bacteria. However, Tuon *et al.* (2010) reported that diabetes mellitus is a risk factor for bacteremia by ESBL-producing *K. pneumoniae* (p=0.043). Silva *et al.* (2006) reported in his study that diabetes mellitus and malignancy are risk factors for infection by ESBL producing bacteria (p=0.02). Diabetes mellitus is a well-known risk factor towards infection. Patients with diabetes have under-optimal immune system function, rendering them susceptible towards infectious agents (Casqueiro *et al.* 2012).

An interesting finding in this study is that the proportion of ESBL-producers among *E. coli* isolates reaches 75%. This result differs from a study of microbial resistance pattern in Dr. Soetomo Hospital by Kuntaman *et al.* (2006). The study reported prevalence of ESBL-producers among *E.coli* was 34.86% and among *K. pneumonia* isolates reaches 35.35%. The study involved larger sample size and isolates from wards other than Internal Medicine; therefore it represented a larger population. Meanwhile Pajariu *et al.* (2010) reported the prevalence of ESBL-*E. coli* was 54.32% and ESBL-*K. pneumonia* was 50% in Dr. Kariadi General Hospital, Semarang. Microbial epidemiology usually varies among health institutes and changes overtime. However, the small sample size involved in this study might cause sampling bias and the results might not accurately describe population characteristics. Changes of bacterial population might also be the case, however no report was available and data on microbial pattern distribution is lacking. Further surveillance is needed to confirm whether there is a changing trend in bacterial distribution in Dr. Soetomo Hospital.

ESBL gene may be harbored in the bacterial chromosome or in plasmid. Severin *et al*. (2009) reported that the most predominant ESBL isolate from Dr. Soetomo Hospital was the producer of CTX-M-15, of which the gene is most commonly harbored in plasmid. Unfortunately, molecular ESBL analysis was not conducted in this study; hence it is not known whether the strains of ESBL-producing bacteria in this study were from clonal spread or horizontal plasmid transfer.

Finally, none of the classes of antibiotics used by patients is found to be a risk factor for infection by ESBL-producing bacteria. Osthoff *et al*. 2015 and Huang *et al*. 2007 reported that the use of third generation cephalosporin is a risk factor for infection by ESBL-producing bacteria. The use of beta lactam-beta lactamase inhibitor (Chopra *et al*. 2015; Harris *et al*. 2007) and fluoroquinolones (Soraas *et al*. 2013) were also found as risk factors for infection by ESBL-producing bacteria. The failure to obtain an association between antibiotic use and infection by ESBL-producing bacteria in this study might be caused by involvement of small sample size. Another reason would be because exposure towards antibiotics in this study is measured as a categorical variable (exposed/unexposed) rather than as a continuous variable such as measuring Defined Daily Dose (DDD), Length of Therapy (LOT) or Duration of therapy (DOT). Schechner *et al*. (2013) reported that an association of antibiotic use with infection by resistant organisms is more likely to be obtained if the antibiotic used was measured as a continuous variable

rather than a categorical variable.

In conclusion, age, gender, pre-infection length of hospital stay, comorbidities and antibiotic use were not risk factors for ESBL-infection was discovered, However, this study finds a significantly larger proportion of ESBL-*E. coli* compared to non-ESBL producing *E. coli*. Further studies should include larger sample size and quantitatively measured antibiotic use.

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