

The Sensitivity Pattern of Extended Spectrum Beta Lactamase-Producing Bacteria Against Six Antibiotics that Routinely Used in Clinical Setting

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Abstract: The validated study of extended-spectrum β -lactamase (ESBL) producing bacteria in Indonesia is scarce. Multi-centre study on susceptibility of ESBLs producers is our point of view. A survey was carried out in three teaching hospitals in Surabaya (Dr. Soetomo), Malang (Dr. Saiful Anwar) and Semarang (Dr. Kariadi). Clinical ESBL-producers were collected in over four months period (January to April 2010) up to 300 strains. The susceptibility against 6 antibiotics below were used as a point of view in analysis. As many as 300 isolates were collected, 140 (Surabaya), 85 (Semarang) and 75 (Malang) respectively. The three most prevalent ESBL producers were: *E. coli* (42.7%), *Klebsiella pneumoniae* (47.3%) and *Enterobacter spp* (7%). The other 9 strains were: *Citrobacter spp*, *Klebsiella oxytoca*, *Proteus mirabilis* and *Serratia spp*. The susceptibility analysis was then performed on the three most prevalent isolates. The sensitivity rate of *E. coli*, *Klebsiella pneumoniae* and *Enterobacter spp* against tested antibiotics were 3%, 4% and 5% for cefotaxim; 91%, 87% and 90% for Amikacin; 27%, 54% and 43% for Ciprofloxacin; 98%, 93% and 100% for Cefoperason-Sulbactam; 100%, 96% and 100% for Meropenem; 95%, 94% and 86% for Fosfomycin. As a conclusion, we found that amikacin, cefoperason-sulbactam, meropenem and fosfomycin, are prospective for empiric therapy in clinical setting of health services where ESBL producing bacteria were prevalent as causative. *J Indon Med Assoc.* 2011;61:482-6.

Keywords: Extended Spectrum Beta Lactamases (ESBL), antibiotic, antimicrobial resistance, sensitivity.

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Pola sensitivitas bakteri penghasil *Extended Spectrum Beta Lactamase* terhadap Enam Antibiotik yang Biasa Dipergunakan pada Pengobatan di Klinik

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Abstrak: Data bakteri penghasil ESBL yang tervalidasi di Indonesia sangat terbatas. Studi multisenter tentang pola dan sensitivitas bakteri penghasil ESBL menjadi kajian utama pada studi tersebut. Telah dilakukan survei di tiga senter Rumah Sakit Pendidikan di Surabaya (RSUD Dr. Soetomo), Malang (RSUD Dr. Saiful Anwar) dan Semarang (RSU Dr. Kariadi). Isolat klinik penghasil ESBL dipisahkan sejak Januari-April 2010 untuk mencapai target sebanyak 300 isolat. Identifikasi dan uji kepekaan dilakukan sesuai standar di Laboratorium Mikrobiologi RSUD Dr. Soetomo Surabaya. Enam antibiotik dijadikan kajian dalam studi tersebut, yakni Sefotaksim, Amikasin, Siprofloksasin, Cefoperason-sulbaktam dan meropenem. Sebanyak 300 isolat berhasil terkumpul, terdiri 140 (Surabaya), 85 (Semarang) dan 75 (Malang). Isolat terdiri dari *E. coli* (42,7%), *Klebsiella pneumoniae* (47,3%) and *Enterobacter spp* (7%). Isolat lain lebih sedikit (*Citrobacter spp*, *Klebsiella oxytoca*, *Proteus mirabilis* dan *Serratia spp*). Pada tiga spesies isolat yang dianalisis menunjukkan bahwa sensitivitas *E. coli*, *Klebsiella pneumoniae* and *Enterobacter spp* terhadap antibiotik uji adalah 3%, 4% and 5% terhadap sefotaksim; 91%, 87% dan 90% for amikasin; 27%, 54% dan 43% untuk siprofloksasin; 98%, 93% and 100% untuk sefoperason-sulbaktam; 100%, 96% dan 100% untuk Meropenem; serta 95%, 94% and 86% untuk Fosfomisin. Sebagai kesimpulan, amikasin, sefoperason-sulbaktam, meropenem dan fosfomisin dapat dipakai sebagai pedoman untuk terapi empirik di rumah sakit dengan insidens bakteri penghasil ESBL tinggi. *J Indon Med Assoc.* 2011;61:482-6.

Kata kunci: Extended Spectrum Beta Lactamases (ESBL), antibiotik, reseistensi antimikroba, sensitivitas.

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Introduction

In two decades, bacterial resistance to β -lactam antibiotics has risen sharply. It is also predicted that spread among patients contributes to the increase of bacterial resistance. The extended-spectrum β -lactamases (ESBLs), enzymes that hydrolyze the expanded-spectrum cephalosporins, like ceftazidime and cefotaxime, and/or the monobactam aztreonam, is one of the most popular 'virulent enzyme.' The first isolate of ESBL producing bacteria was reported in Germany in 1983. Subsequently, many ESBLs, predominantly of SHV and TEM variants, have been reported in clinical isolates.¹ Many type of ESBL enzymes have been identified. In recent years, cefotaximases of the CTX-M type have become a predominant cause of higher levels resistance.²

Since first identified at the beginning of 1980s, extended-spectrum β -lactamases (ESBL)-producing microorganisms, mainly to the family of *Enterobacteriaceae*, have spread by nosocomial routes throughout the world. As has been dem-

onstrated for *Klebsiella pneumoniae*, prevalence in a particular hospital may vary from 0% to approximately 50%, and nationally, in some countries, it may reach approximately 15%. The ESBL-encoding genes are usually located on large conjugative plasmids, which also often carry genes responsible for resistance to other antibiotics.^{3,4} The conjugative plasmid that carry many genes encoding for antimicrobial resistance, have also found in Dr. Soetomo Hospital, Surabaya.⁵

Extended-spectrum β -lactamases (ESBLs) are a group of plasmid-borne enzymes with the ability to hydrolyse third-generation cephalosporins and monobactams. Most organisms harboring such enzymes remain susceptible to carbapenems, whereas the activity of ciprofloxacin, cefepime, and beta-lactam/beta-lactamase inhibitor combinations is variable. Infections caused by microorganisms producing ESBLs have become a serious problem for hospitals worldwide.⁶

This study will explore the susceptibility pattern of ESBL producing microorganisms against 6 antibiotics that are commonly used in clinical setting in Indonesia.

Methods

This was a descriptive study to reveal the susceptibility pattern of ESBL-producing bacteria against 6 commonly used antibiotics. ESBL producing bacteria was collected from clinical isolates that were sent to microbiology laboratory in 3 teaching hospitals: Dr. Soetomo Hospital (Surabaya), Dr. Kariadi Hospital (Semarang) and Dr. Saiful Anwar Hospital (Malang). Identification of bacteria and phenotypic confirmation of ESBL producer was conducted by two methods. First, we used double-disk synergy test using disks of amoxicillin-clavulanic acid, cefotaxime and ceftazidime.¹⁷ Secondly, we used Phoenix[®] machine according to the instruction of manufacturer.

Susceptibility test against targeted antibiotics was further conducted using diffusion method.⁷ It is due to the absence of one or more antibiotics pannels in the reagents provided in automatic machine (Phoenix[®] or equivalent) and these antibiotics are commonly used in clinical setting. The six tested antibiotics were cefotaxime, meropenem, fosfomycin, cefoperazone-sulbactam, amikacin and ciprofloxacin. *Escherichia coli* ATCC 25922 was used as a control for susceptibility testing. Interpretation of susceptibility test was performed according to CLSI, 2011.⁷

Table 1. Distribution of ESBL Producing Bacteria Among Centres, Surabaya, Semarang and Malang

Microorganisms	Origin			Total (%)
	Surabaya	Semarang	Malang	
<i>Citrobacter spp</i>	2	1	0	3 (1%)
<i>Enterobacter spp</i>	4	13	4	21 (7%)
<i>Escherichia coli</i>	61	36	31	128 (42.7%)
<i>Klebsiella oxytoca</i>	1	1	1	3 (1%)
<i>Klebsiella pneumoniae</i>	71	32	39	142 (47.3%)
<i>Proteus mirabilis</i>	1	0	0	1 (0.3%)
<i>Serratia spp</i>	0	2	0	2 (0.7%)
Total	140	85	75	300 (100%)

Table 3. The Sensitivity Pattern of ESBL Producing Bacteria Isolated from Three Centres, Dr. Soetomo Hospital Surabaya, Dr. Kariadi Hospital Semarang and Dr. Saiful Anwar Hospital Malang

Bacteria	Total	<i>E. coli</i>		Total	<i>K. pneumoniae</i>		Total	<i>Enterobacter spp</i>	
		Sens	Sens%		Sens	Sens%		Sens	Sens%
Cefotaxime	128	4	3.22	142	6	4.23	21	1	4.8
Meropenem	128	128	100	142	137	96.5	21	21	100
Fosfomycin	128	122	95.31	142	134	94.4	21	18	85.7
Cefo-Sulb	128	125	97.66	142	134	94.4	21	21	100
Amikacin	128	116	90.62	142	123	86.6	21	19	90.5
Ciprofloxacin	128	34	26.56	142	76	53.5	21	9	42.9

Note: Sens=Sensitive; cefo-sulb = cefoperazone-sulbactam combination. All sensitive and intermediate result were included in sensitive result.

Results

From January 2010 to April 2010, a total of 300 ESBL producing isolates have been collected with three most prevalent ESBL producers were *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter spp* as seen in Table 1.

The isolates were collected from various specimen as described in table 2. Specimen data from Semarang was not available.

The analysis of susceptibility was performed only on the three most prevalent bacteria, as seen in Table 3.

The intermediate result of susceptibility against cefoperazone-sulbactam and amikacin were high, mainly in two prevalent isolates, *E. coli* and *K. pneumoniae*. The intermediate result of cefoperason-sulbactam were 25.8% (33 isolates) in *E. coli* and 35.9% (51 isolates) in *K. pneumoniae*, and amikacin were 13.3% (17 isolates) and 9.9% (14 isolates) for *E. coli* and for *K. pneumonia* respectively.

Discussion

The sensitivity rate of cefotaxim and ciprofloxacin *in vitro* were low and were not effective for clinical use as the treatment of ESBL-producing bacteria. The sensitivity of other four antibiotics, namely meropenem, fosfomycin, amikacin and combination cefoperazone-sulbactam were still potential for clinical usage to treat ESBL- producing bacteria.

Table 2. The Origin of the Specimen of ESBL Producer Bacterial Isolates from Surabaya and Malang

Specimen	Surabaya		Malang		Total	
	Total	%	Total	%	Total	%
Urine	36	25.7	21	28	57	26.5
Blood	24	17.1	13	17.3	37	17.2
Pus	41	29.3	16	21.3	57	26.5
Sputum	20	14.3	17	22.7	37	17.2
Faeces	11	7.9	3	4	14	6.5
Others	8	5.7	5	6.7	13	6.1
Total	140	100	75	100	215	100

Notes: Others=pleural fluid, wound swab, tissue, fistule swab (the data of specimen from Semarang isolates were not available)

Table 4. The Intermediate Result of Susceptibility Test in *E. coli*, *K. pneumoniae* and *Enterobacter spp.* Against Fosfomycin, Cefoperason-sulbactam, Amikacin and Ciprofloxacin

Bacteria	<i>E. coli</i>			<i>K. pneumoniae</i>			<i>Enterobacter spp</i>		
	Total	Sens (%)	Inter (%)	Total	Sens (%)	Inter (%)	Total	Sens (%)	Inter (%)
Fosfomycin	128	116 (90.6)	6 (4.7)	142	125 (88)	9 (6.3)	21	14 (66.7)	4 (19.1)
Cefo-Sulb	128	92 (71.9)	33 (25.8)	142	83 (58.5)	51 (35.9)	21	12 (57.1)	9 (42.9)
Amikacin	128	99(77.3)	17 (13.3)	142	109 (76.8)	14 (9.9)	21	15 (71.4)	4 (19.1)
Ciprofloxacin	128	27 (21.1)	7 (5.5)	142	54 (38)	22 (15.5)	21	7 (33.3)	2 (9.5)

Note: cefo-sulb=Cefoperazone-Sulbactam; Sens = Sensitive; Inter = Intermediate result in susceptibility test. The total quantity of *Enterobacter* is too small for further analysis.

The sensitivity profile of clinical isolates of ESBL-producing bacteria from Surabaya, Semarang and Malang is consistent with the current worldwide situation, mainly meropenem.⁸⁻¹² Meropenem is a reserved antibiotic in clinical setting, and of course fosfomycin, cefoperason-sulbactam and amikacin, are three prospective candidates of antibiotics wedas an alternative drugs in clinical usage for combating ESBL's producers. Even clinical judgement is very important for drug selection, mainly in seriously ill patient. As many studies indicates, cefotaxim and ciprofloxacin are not effective for clinical usages. Clinical and Laboratory Standard Institute (CLSI), 2011⁷ has also recommended that the third generation cephalosporins should be excluded as an anti-infective drug for ESBL producers, even if the result of Microbiological culture is sensitive. The author think that this issue need to be studied further for definitive therapy. Ciprofloxacin has a certain concern, even though it is not in similar group of cefotaxim, many studies showed that the ESBL producers were mostly resistant against ciprofloxacin. It is predicted by two aspects, the tendency of Health practitioner to change the resistant third generation cephalosporin to ciprofloxacin, and also the resistant trait encoded by plasmid that in combination with other antibiotics, including third generation of cephalosporin, and it transfers to the other bacteria in hospital setting.^{13,14} In surveillance about ESBL producers in Dr. Soetomo Hospital, since January to November 2011 showed that the rate of ESBL producer strain among *E. coli* was 45.32% (329 of 726 total isolates) and *K. pneumoniae* was 50.28% (360 of 716 isolates).¹⁵ It means that the problem of ESBL in this hospital is increasing, and the antibiotic policy for increasing the prudent use of antibiotic, mainly third ge-generation cephalosporin, is absolutely needed.

The further analysis of 5 strains of meropenem-resistant *K. pneumoniae* showed that 3 strains were sensitive against fosfomycin and cefoperazone-sulbactam, and 2 strains were sensitive against amikacin. This suggested that fosfomycin, amikacin and cefoperazone-sulbactam could be an alternative drug for meropenem resistant microbes. In common clinical setting, meropenem is the first choices for containment of ESBL producer. This study showed that the other alternative antibiotic would be the choices, in case of

resistant against meropenem. However, higher rate of intermediate result should raise an awareness for the possibility of resistance development in the future.

The two prevalent of specimen were urine and pus, followed by blood and sputum. Therefore, urinary tract infection and wound infection should raise special concern because ESBL producers were mainly caused by nosocomial infection. Thus, universal precaution principle should be put in higher priority during in-patient management, especially for patients using urethral catheter and/or with exposed wound. Lower respiratory tract infection should also require special attention, especially for patient with endotracheal devices.

Conclusions

ESBL's producing bacteria are spread among species of *Enterobacteriaceae*, the two most frequent were *Escherichia coli* and *Klebsiella pneumoniae*, then followed by *Enterobacter spp* and others. The four main specimen of origin of the ESBL producers were urine, pus, sputum and blood. Four antibiotics were still potential for clinical usage: amikacin, meropenem, fosfomycin and combination cefoperazone-sulbactam. The meropenem-resistant strains were still sensitive against fosfomycin, amikacin and cefoperazone-sulbactam. The higher rate of intermediate result for cefoperason-sulbactam and, in the lesser extend, for amikacin, need special attention in clinical usage, in the context of the prevention of the development of resistant strains in the future.

Transparency declarations

None to declare.

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