Correlation of *Bla*$_{shv}$ And *Bla*$_{tem}$ Genes in Extended-Spectrum Beta-Lactamase (Esbl) - Producing *Acinetobacter Baumannii* With Patient's Outcome at Dr. Wahidin Sudirohusodo Hospital, Makassar

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Abstract

Acinetobacter baumannii causes the high morbidity and mortality. *Acinetobacter baumannii* has ESBL enzyme that makes it resistant to the mostly beta-lactam antibiotics. Some ESBL – coding genes are *bla*$_{SHV}$ and *bla*$_{TEM}$. The purpose of this study was to evaluate correlation *bla*$_{SHV}$ and *bla*$_{TEM}$ genes in Extended-Spectrum Beta-Lactamase (ESBL) - producing *Acinetobacter baumannii* with outcome (length of stay and death) at Dr. Wahidin Sudirohusodo Hospital, Makassar. The study used cross-sectional method conducted on *Acinetobacter baumannii* isolates and medical record at Dr. Wahidin Sudirohusodo, Makassar. ESBL was detected with automated culture, *bla*$_{SHV}$ and *bla*$_{TEM}$ genes was detected with PCR. Statistical analysis used Chi Square, Fisher Exact, Independent-t and Pearson's Correlation tests. Significance of test if $p<0.05$. Research of 57 *Acinetobacter baumannii* isolates, ESBL was found in 20 isolates (35%). Average length of stay (LOS) in positive ESBL (26.3 days) was significantly longer than in negative ESBL (16,5 days)(p = 0,016). Mortality rate was higher in positive ESBL (55%) than negative ESBL (35,1%), but statistically not significant ($p = 0.147$). The *bla*$_{TEM}$ gene found in 53 isolates (93%) and there was no isolate bringing *bla*$_{SHV}$ gene. In positive ESBL, average length of stays (LOS) in positive *bla*$_{TEM}$ was 25.4 days while in negative *bla*$_{TEM}$ was 42 days; Mortality rate was higher in positive *bla*$_{TEM}$ compared to negative *bla*$_{TEM}$ (57.9%, 0.0%, consecutively), but statistically not significant ($p>0.05$). It was concluded that *bla*$_{TEM}$ gene was found 93% and *bla*$_{SHV}$ was not found. The LOS in positive ESBL was significantly longer.

***Keywords**: Acinetobacter baumannii, ESBL, bla$_{SHV}$, bla$_{TEM}$, Outcome*

Introduction

Acinetobacter baumannii is an opportunistic bacterial pathogen primarily associated with a high morbidity and mortality.[1,2] It is an important agent of nosocomial infections worldwide, such as urinary tract infections, septicemia, pneumonia, burns, meningitis, and wound infections in hospitals.[3-5] *Acinetobacter baumannii* infection causes duration of stay in hospital becomes longer, increases burden of cost and difficult to treat.[6] *World Health Organization* (WHO) had published list of bacterias that needed urgently new antibiotics in 2017 and placed *Acinetobacter baumannii* in the first row critical priority group. National Nosocomial Infections Surveillance (NNIS) System found that there was an significant increase *Acinetobacter* infection between 1987 and 1996 in United State of America about 54%.[7] Studies in Indonesia showed that one of pathogens in late onset Hospital Acquired Pneumonia infection was *Acinetobacter baumannii* with prevalence 23,2%.[8,9]. Data in 2019 at Wahidin Sudirohusodo Hospital Makassar showed prevalence *Acinetobacter baumannii* infection about 19,6% of all Gram-negative bacterial infections in recent last eight months. Resistance mechanism of *Acinetobacter baumannii* can be through several ways such as production enzyme that able to hydrolyze drugs component, drug *efflux*, failure to reach target or change in drug target.[10,11]

Extended-spectrum beta-lactamase (ESBL) is an enzyme that has resistance to the most beta-lactam antibiotics including penicillin, cephalosporin and

aztreonam monobactam.[12,13] *Acinetobacter baumannii* is one of pathogens that capable to produce ESBL. Genes that encodes ESBL is located in plasmid that is easily transferred to another pathogen and therefore resistence is spreading.[14,15] First publication about genes that encoding beta-lactamase was found in 1983. Some of these genes are *Temoniera* (bla_{TEM}) and *sulfhydril variable* (bla_{SHV}). Prevalence bla_{SHV} gene in China 30,7% during 1998-1999. Korea, Japan, Malaysia and Singapore reported prevalence 5-8% while Thailand, Taiwan, Filipina and Indonesia reported prevalence 12-24% with bla_{SHV} gene was the dominant gene.[14] Enzymes of ESBL that are resistant to the third generation cephalosporin antibiotics are encoded by bla_{SHV} gene 58%, bla_{TEM} gene 20% and *Cefotaxime Munich* (bla_{CTX-M}).[10] The purpose of this study was to evaluate correlation bla_{SHV} and bla_{TEM} genes in Extended-Spectrum Beta-Lactamase (ESBL) - producing *Acinetobacter baumannii* with patient's outcome at Dr. Wahidin Sudirohusodo Hospital, Makassar.

Method

This study is a cross sectional study detecting bla_{SHV} and bla_{TEM} genes in ESBL producing - *Acinetobacter baumannii* isolates and evaluating correlation these genes with outcome length of stays and mortality. The study was conducted during August 2019 at Clinical Pathology Laboratory of Dr. Wahidin Sudirohusodo Hospital and Microbiology laboratory of Hasanuddin University, Makassar, Indonesia. The study sample was all *Acinetobacter baumannii* isolates that had been confirmed using Vitek 2 Compact. Contamined samples were excluded. All isolates were tested for ESBL and Polymerase Chain Reaction for bla_{SHV} and bla_{TEM} genes.

Data were analyzed statistically by *Chi Square*, *Fisher Exact*, *Independent-T* and *Pearson's* tests using SPSS version 25. The results were considered significant if $p<0.05$.

Results

Table 1. General Characteristics

Variables		***n***	**%**	***Mean±SD***
Age (years)	<20	12	21,1	
	20-39	9	15,8	
	40-59	23	40,4	
	>=60	13	22,8	
Sex	Men	38	66,7	
	Women	19	33,3	
ESBL	Positive	20	35,1	
	Negative	37	64,9	
bla_{SHV}	Positive	0	0	
	Negative	57	100	
bla_{TEM}	Positive	53	93	
	Negative	4	7	
Death	Yes	24	42,1	
	No	33	57,9	
Length of Stay(days)				20±15

The study samples obtained were 57 isolates which met the inclusion criteria. The characteristics of study samples can be seen in Table 1 that shows male subjects were more than female. Most study subjects were found in the 40-59 year group. PCR of bla_{SHV} and bla_{TEM} of the study are shown in Figure 1.

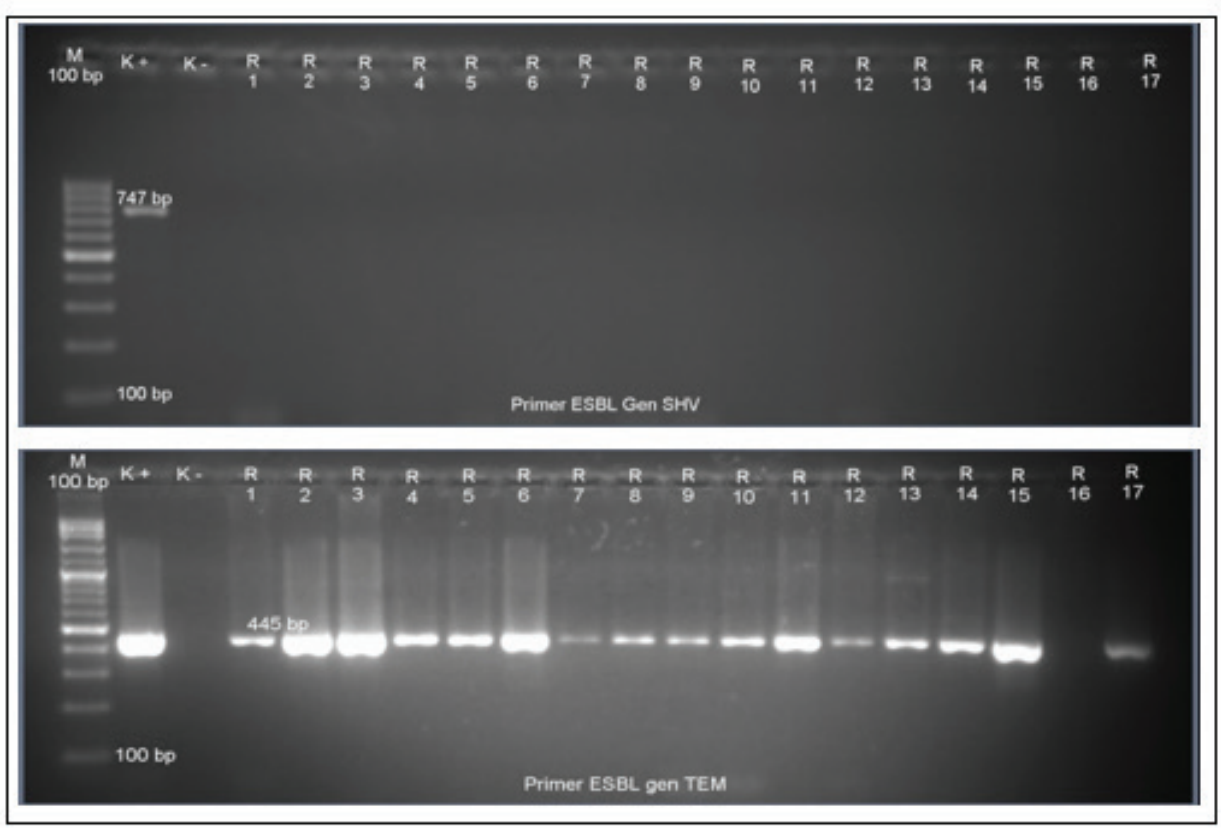

Figure 1. PCR of bla_{SHV} and bla_{TEM} of the study.

Table 2. Correlation Length Of Stay Based On ESBL

ESBL	*n*	LOS(days) *Mean±SD*	*p
Positive	20	26,3±17,5	0,016
Negative	37	16,5±11,9	

**Independent-t test*

Table 2 showed the mean length of stay in positive ESBL was higher than in negative ESBL. Statistical analysis showed significant correlation between ESBL and LOS (p = 0.016).

Table 3. Correlation Mortality Based On ESBL

ESBL		Mortality		*p
		Yes	No	
Positive	*n*(%)	11(55)	9(45)	0,147
Negative	*n*(%)	13(35,1)	24(64,9)	

**Chi Square test*

Table 3 showed that mortality rate was higher in positive ESBL (55%) than in negative ESBL (35,1%), but statistical analysis result was not significant (p>0,05).

Table 4. Comparison bla_{TEM} Based On Outcome

Group			N	Outcome				
				LOS (days)		Mortality		
				(mean±SD)	*p	Yes n(%)	No n(%)	**p
ESBL								
	bla_{TEM}	Pos	19	25,4±17,6	#	11 (57,9)	8 (42,1)	0,450
		Neg	1	42,0±17,6		0 (0)	1 (100)	
NonESBL								
	bla_{TEM}	Pos	34	17,0±12,2	0,439	12 (35,3)	22 (64,7)	1,000
		Neg	3	11,3±7,8		1(35,3)	2 (66,7)	

*Independent-t test
**Fisher Excat test
Could not be analyzed because negative bla_{TEM} was only one isolate

*Independent-t test

**Fisher Excat test

Could not be analyzed because negative bla_{TEM} was only one isolate

Table 4 showed that presentation mortality in ESBL group was found higher in positive bla_{TEM} (57,9%) than in negative bla_{TEM}, but statistical analysis was not significant (p>0,05). Presentation mortality in nonESBL group was found higher in positive bla_{TEM} (35,5%) than in negative bla_{TEM}(33,3%), but statistical analysis was not significant (p>0,05).

Discussion

Acinetobacter baumannii is an opportunistic Gram-negative pathogen that is able to produce ESBL. Extended-spectrum β-lactamases are the main cause of bacterial resistance to beta-lactam antibiotics. In this study, positive ESBL was found 35% of all *Acinetobacter baumannii* isolates. The results obtained in this study were not consistent with Abdar *et* al in Iran that found 59% of 100 *Acinetobacter baumannii* were positive.[16] Sharif *et al* also found detection rate ESBL was 51%.[17] This difference was caused by the high use of the third generation cephalosporine antibiotics in hospital and home care in that country.[16] Overuse antibiotics and *hand hygine* were factors that caused adalah higher incidence ESBL in hospital setting. Abdar *et al* and Sharif *et al* used diffusion test agar while our study used automated culture method.[16,17] Automated culture method has sensitivity 98,1% and specificity 99,7%. The method needs 6 - 13 hours (mean 8.2 hours) and therefore this method can be a choice for detecting isolates of ESBL producing- bacteria.[18]

This study found significant difference statistically between length of stay and positive ESBL and negative ESBL. *Acinetobacter baumannii* that has ESBL causes antibiotic resistance and therefore infection is difficult to treat and duration stays in hospital is longer and increase burden of hospitality costs. The results obtained in this study found that mortality in *Acinetobacter baumannii* was high (42%). Higher mortality rate was found in isolates with positive bla_{TEM} and also found higher in positive ESBL although statistical analysis is not significant. *Acinetobacter baumannii* is the most pathogen from *Acinetobacter* genus. This pathogen often causes infection in compromised subject, especially in ICU with invasive support. *Acinetobacter baumannii* causes *ventilator associated pneumonia* (VAP), meningitis, septicemia, urinary track infection, and burn infection.[17] *Acinetobacter baumannii* infection causes severe sepsis. High mortality also increases with invasive procedure such as mechanical ventilator and central venous catheter.

Acinetobacter baumannii produces ESBL through role of plasmid such as bla_{SHV} and bla_{TEM} genes. The study which we did in Wahidin Sudirohusodo Hospital revealed that there was no isolate containing bla_{SHV} gene while bla_{TEM} gene dominated most of all samples (93%). The results obtained in this study were consistent

with Abdar *et al* who did not find any bla_{SHV} gene in ESBL producing-*Acinetobacter baumannii* isolates.[16]. Koo *et al* also did not find any bla_{SHV} gene in Korea.[19] The result of this study were consistent with Chaudhary *et al* who found prevalence of bla_{TEM} in positive ESBL was 87%.[20] Adams-Haduch *et al*, also found prevalence bla_{TEM} gene about 73,5%.[21] The results obtained in this study were different with Sharif *et al* who found 56% bla_{TEM} and 63% bla_{SHV} of ESBL isolates.[17] Other studies in the world show variation result. The difference of our study and others indicates that type of ESBL genes can be variable from one place to another place. Bla_{TEM} gene that encodes ESBL is located in integron 1 of plasmid and therefore that gene is easily tobe transferred from one *Acinetobacter baumannii* to another *Acinetobacter baumannii*.[16] Genotype detection method used PCR technique that has high specificity and sensitivity therefore this technique was able to detect 93% bla_{TEM} gene in *Acinetobacter baumannii* isolates in this study. The difference between prevalence of bla_{TEM} gene and ESBL in this study could be caused by *Acinetobacter baumannii* had already had bla_{TEM} resistant gene in the plasmid but this gene was not transferred yet to the chromosome or the bla_{TEM} resistance gene had been in the chromosome but synthesis ESBL was not happened. Bla_{TEM} gene that was found in *Acinetobacter baumannii* isolates indicates that this gene can be a candidate for molecular skrining from positive ESBL samples in our hospital and can be an important concern because that *Acinetobacter baumannii* can further produce ESBL if antibiotics are not used rationally.

This study had some limitations. This study did not analyze the type and duration antibiotics that had been used by the patients and also diagnosis of the disease. These factors might cause bias for length of stay in this study.

CONCLUSION AND SUGGESTION

This study concluded that there were 35% positive ESBL of all *Acinetobacter baumannii* isolates. Bla_{SHV} gene was not found and bla_{TEM} gene was dominant 93%. Prevalence positive ESBL was higher in positive bla_{TEM} gene. Length of stay positive ESBL was significant statistically in positive ESBL. Mortality rate was high (42%) in *Acinetobacter baumannii* infection. We also recommend further study that evaluates factors related to the outcome (length of stay and mortality) in subjects with ESBL producing *Acinetobacter baumannii* infection.

Ethical Clearance – Taken from Health Study Ethical Committee of Hasanuddin University, Medical Faculty, Dr. Wahidin Sudirohusodo Hospital, Makassar

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Conflict of Interest – The authors declare that they have no conflict of interest.

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