



Original Research

THE CARRIER RATE OF EXTENDED SPECTRUM BETA LACTAMASE (ESBL) PRODUCING BACTERIA IN COCKROACHES (*PERIPLANETA AMERICANA*) IN HOSPITAL AND COMMUNITY

Ardhiya Puspita¹, Radita Yuniar Arizandy², Eddy Bagus Wasito³,  Kuntaman^{2,4} 

¹Postgraduate Program of Basic Medical Science, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

²Institute of Tropical Disease, Universitas Airlangga, Surabaya, Indonesia

³Department of Clinical Microbiology, Faculty of Medicine, Universitas Airlangga, Surabaya, Indonesia

⁴Indonesian Society for Clinical Microbiology, Indonesia

ABSTRACT

Cockroach (*Periplaneta americana*) is one of the vectors in the environment that can transmit disease. Cockroaches can act as potential mechanical vectors of antibiotic resistant bacteria. Enterobacteriaceae is a gram-negative bacteria that has natural habitats in the digestive tract of humans and animals. Enterobacteriaceae that produce Extended Spectrum β -lactamases (ESBLs) have emerged as major pathogens in hospitals. The study analyzed the prevalence of ESBL producing bacteria in cockroaches that lived in hospitals and residential homes. In this study, a total of 200 cockroaches consisting of 100 cockroaches from the hospital environment and 100 cockroaches from the residential environment were analyzed bacteriologically for colonization of ESBL producing Enterobacteriaceae. The specimen of the alimentary tract was taken and sub-cultured in MacConkey agar supplemented with cefotaxime 2 ug/ml. Growth colonies were suggested as an ESBL-producing bacteria, then were confirmed as ESBL producers by the Double Disk Synergy Test (DDST). The ESBL gene was detected by Polymerase Chain Reaction (PCR). Among 100 household cockroach samples, 14 (14%) were identified as ESBL producers, while 100 hospital cockroaches were 26 (26%) positive ESBL. The ESBL gene, in hospital cockroach were identified of CTXM 19 (19%), SHV 7 (7%), and not any TEM gene, while among household cockroaches were identified CTXM 2 (2%), SHV 11 (11%), and also not detected TEM ESBL gene. Among ESBL genes, only the CTXM gene was significantly different between household and hospital cockroaches.

Keywords: ESBL; *E coli*; cockroach; disease

Correspondence: Kuntaman, Clinical Microbiology, Faculty of Medicine, Universitas Airlangga=Dr. Soetomo General Academic Hospital, Surabaya=Indonesian Society for Clinical Microbiology, Indonesia. Email: kuntaman@fk.unair.ac.id

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1. Bacteriologically for colonization of ESBL producing Enterobacteriaceae in cockroaches (*Periplaneta americana*) were analyzed.
2. The prevalence of ESBL producing bacteria among cockroaches in hospitals is bigger than in households.

INTRODUCTION

Cockroaches (*Periplaneta americana*) are known as an insect living in dirty environments at housing, restaurants and even hospitals, where they eat a variety of waste substances that possibly inhabited by any bacteria. They can passively transmit microbial pathogens including *Salmonella*, *Campylobacter*, *Shigella*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* to humans when they eat food scraps. However, cockroaches that colonize this environment can also act as potential mechanical vectors of antibiotic resistant bacteria (Loucif et al. 2016, Gwenzi et al. 2021).

Enterobacteriaceae is the bacteria that most often causes diseases, such as urinary tract infections (UTI), pneumonia, septicemia, cholecystitis, cholangitis, peritonitis, gastroenteritis and meningitis. Enterobacteriaceae is a gram-negative bacterium that has natural habitats in the digestive tract of humans and animals, and also the environment. Enterobacteriaceae that produces Extended Spectrum β -lactamases (ESBLs) has emerged as major pathogens in hospitals. The gene was first reported in the mid-1980s, mainly found in *Klebsiella pneumoniae* and *Escherichia coli* (Bradford 2011, Kuntaman et al. 2011). ESBL-

producing Enterobacteriaceae is also present in animals, in patients and populations in the community, with and without any diseases or chronic conditions (Mirelis et al. 2013).

ESBL producing, especially *Klebsiella pneumoniae* and *Escherichia coli*, are mostly resistance against any antibiotics, mainly the third generation cephalosporins, and aztreonam, where the enzyme activity can be inhibited by β -lactamase inhibitors, such as clavulanic acid. The ESBL encoding genes are in a plasmid that is easily transmitted to the other germs resulting in the spread of resistance (Paterson et al. 2010). The third-generation cephalosporin, which was marketed in the 1980s, was originally intended to overcome the β -lactamase producing germ, and widely used due to its less toxic effect. Nevertheless, its wide use impacted the increasing and spread of ESBL resistant genes among Gram negative (Park 2014, Ruppé et al. 2015).

ESBL-producing bacteria was also reported by Reich et al (2013) in chicken carcasses of about 88.6% cases and in cloaca of chicken of about 72.5% samples. Most of these bacteria were *E. coli*. The study of Salviati et al (2014) showed a high prevalence of ESBL-producing *E. coli* from the environment faecal waste storage of about 47.6%, and 5.9% of pig cage swabs.

Resistant bacteria in the environment can spread to humans through contaminated food and water or through direct contact with animals or humans. In the study of Mesa (2006), ESBL-producing bacteria in food samples of salads (tomatoes and lettuce) and cooked foods by 0.4%, and in waste disposal system is almost 100%. Aycan et al (2013) also reported that hospital waste was found in 89% of ESBL-producing *E. coli* strains.

Organisms that produce ESBL also carry genes that provide resistance to non-beta lactams including quinolones, aminoglycosides, tetracyclines and sulfonamides. This is an antibiotic resistance enzyme that is often found in members of Enterobacteriaceae including *Escherichia coli* and *Klebsiella pneumoniae* as well as non-enteric bacteria, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The selection pressure that drives the evolution of ESBL has been associated to the intensive use of oxyimino-beta lactam, broad spectrum antibiotics, prolonged hospitalization, medical devices and severe illness (Lin et al. 2010).

These organisms can also transmit to humans through fomites and other non-human hosts, such as cockroaches, and in such circumstances, they cause infection. Since arthropods (i.e., cockroaches) can contaminate human food by carrying agents of various diseases with the risk of human infection in hospitals

and in the community, it is therefore important to take cockroaches seriously into consideration for detecting bacteria that contain drug-resistant genes, such as ESBL resistant gene.

MATERIALS AND METHODS

The design of this study was observational analytic-comparative with cross sectional study. The sampling technique used was consecutive sampling which required the inclusion criteria of the research sample. In this study, a total of 200 cockroaches consisted of 100 cockroaches from the hospital environment, and 100 cockroaches from the residential environment. The cockroaches were picked randomly from different parts of the house, like the kitchen, bathroom, guest room, storage, gutter, and exhaust of the house. Cockroaches (*Periplaneta americana*) were picked by using insecticide-spray and taken using sterile gloves. Then, every cockroach is put into a sterile pot.

Furthermore, the cockroaches were soaked in 70% alcohol for 5 minutes to clean the contaminant in the cockroach surface. After that, the alcohol was thrown away, and the sterile 0.9% physiological saline was added for 5 ml then vortexed. The next step was that the cockroaches were picked using sterile pincers and put into a glass plate for dissecting the cockroach visceral. The cockroach alimentary tract was dissected, then put out the inside organ and kept in the 5 ml of 0.9% physiological saline, and finally let it for 10-20 minutes for a homogenate bacterial test.

The identification of ESBL producing bacteria

The homogenate sample was inoculated in MacConkey Agar plate supplemented with Cefotaxime (CTX) 2 mg/L. and incubated 37 °C for 18 – 24 hours. The growth colonies were suspected as ESBL producers, and the ESBL confirmation was conducted by using DDST. Then, if ESBL bacterial production was proven, the biochemical test for bacterial identification was applied (Nakayama et al. 2012).

The growth colonies were taken 4-5 colonies, and suspended in peptone water, diluted until the turbidity equal to McFarland 0.5, then inoculated spread evenly on MuellerHinton agar; keeping it dry for 15 minutes, then put Amoxicillin-Clavulanic Acid (AMC) disc (30/10 ug) in the center of the plate. Other cephalosporin disc was located around the AMC in distance at 15 mm from AMC. The increasing inhibition zone in area between cephalosporin disc and clavulanic acid were interpreted as ESBL producing bacteria.

Bacterial identification

The biochemical test was applied to identify bacterial species of ESBL producers. The biochemical tests were oxidation, sulfide indole motility (SIM), Methyl-red Voges-Proskauer (MR-VP), citrate, urea, and triple sugar iron agar (TSIA). All the biochemical tests and identification according to Bailey and Scotts Diagnostic Microbiology 13th Edition (Tille 2014).

ESBL gene detection

The Polymerase Chain Reaction (PCR) assay to the detection gene of TEM, SHV and CTX-M. The DNA extraction was done by boiling method as a procedure (Ferreira et al. 2011). One bacterial colony was suspended into a 100 µl sterile TE buffer. The suspension was then heated at 100°C temperature for 5 minutes, and centrifuged at 10,000 rpm for 5 minutes. Supernatant was taken as aDNA template, stored at -20°C until used.

PCR was run at each of ESBL gene with total 25µl PCR mix, consisted of 12.5 µl GoTaq Green Master Mix 2x, 1 µl primer forward (blaCTX-M, blaSHV, blaTEM), 1 µl primer reverse (blaCTX-M, blaSHV, blaTEM), 5 µl DNA template and 5.5 µl distilled water (Ferreira et al. 2011).

BlaTEM gene amplification: denaturalised under 96°C for 5 minutes followed by 35 cycles in 96°C for 5 minutes, annealing in 58°C for one minutes and extension in 72°C for 1 minutes and final extension in 72°C for 10 minutes. The used primer TEM-F 5'-ATGAGTATTCAACATTTCCG-3' and TEM-R 5'-CTGACAGTTACCAATGCTTA-3'. The amplicon result was 867 bp.

Amplification of gene blaSHV: denatured in 96°C temperatures for 5 minutes followed by 35 cycles in

96°C for one minute, annealing in 60°C for one minute, and extension in 72°C for one minute and final extension in 72°C for 10 minutes. Primer used were SHV-F5'-GGTTATGCGTTATATTCGCC-3' and SHV-R 5'-TTAGGTTGCCAGTGCTC-3'. The amplicon result was 867 bp.

Amplification of gene blaCTX-M: denatured in 94°C for 7 minutes followed by 35 cycles in 94°C for 50 second, annealing in 50°C for 40 second, and extension in 72°C for one minute and final extension in 72°C for 5 minutes. Primer used were CTX-M-F 5'-ATGTGCAGYACCAGTAARGT-3' and CTX-M-R 5'-TGGGTRAARTARCT SACCAGA -3'. The product of amplicon results was 593 bp. The PCR product was visualized in 1.5 % agarose gel. Electrophoresis was conducted on 100 volts for ± 60 minutes, then stained Ethidium Bromida (0.5 µg/ml).

Data analysis

The result of the research was presented in the table. Data analysis was done by using Chi Square test (if it was eligible), if it was not, Fisher Exact test was applied. The statistic test was SPSS version 22.

RESULTS

Among 100 samples of house cockroaches, 14 (14%) were identified as the ESBL producing bacteria, while hospital cockroaches 26 (26%). The total 14 ESBL producing bacteria from household cockroach, consisting of *Escherichia coli* 8 (8%), *Klebsiella spp* 1 (1%), *Pseudomonas aeruginosa* 1 (1%), *Acinetobacter Spp* 1 (1%), *Klebsiella pneumoniae* 3 (3%), while 26 hospital ESBL producing bacteria were consisting of *Escherichia coli* 9 (9%), *Citrobacter spp* 5 (5%), *Acinetobacter spp* 8 (8%), and *Klebsiella pneumoniae* 4 (4%) (Table 1).

Table 1. Distribution of bacteria ESBL-producing on household cockroaches' samples and hospital samples

No.	Bacterial Types	Numbers n (%)		Total of bacterial types
		Household cockroaches	Hospital cockroaches	
1.	<i>Escherichia coli</i>	8 (8%)	9 (9%)	17
2.	<i>Klebsiella spp</i>	1 (1%)	0 (0%)	1
3.	<i>Citrobacterspp</i>	0 (0%)	5 (5%)	5
4.	<i>Pseudomonas aeruginosa</i>	1 (1%)	0 (0%)	1
5.	<i>Acinetobacterspp</i>	1 (1%)	8 (8%)	9
6.	<i>Klebsiella pneumoniae</i>	3 (3%)	4 (4%)	7
7.	Non ESBL bacterial	86 (86%)	74 (74%)	160
	Total samples	100 (100%)	100 (100%)	200

Table 2. The distribution of ESBL genes of house and hospital cockroaches

Location	CTX-M		SHV		TEM	
	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)	Positive n (%)	Negative n (%)
Household (n=100)	2 (2%)	98 (98%)	11 (11%)	89 (89%)	0 (0%)	100 (100%)
Hospital (n=100)	19 (19%)	81 (81%)	7 (7%)	93 (93%)	0 (0%)	100 (100%)
Total gene	21	179	18	182	0	200 genes

The result of ESBL gene showed that TEM gene was not found in household cockroaches and hospital cockroaches. From 14 of house ESBL producers, 7 (7%) SHV genes and 19 (19%) CTXM were identified. In this study, the SHV gene was mostly found in household samples. It was possible, because the SHV gene was infected through food (Greko et al. 2017). SHV variants were detected for the first time in Switzerland with blaSHV-12 (Table 2).

DISCUSSION

ESBL-producing bacteria in cockroaches (*Periplaneta americana*) on residential environment were mostly found in cockroach of gutter 11 (11%) and 3(3%) were found inside of the house, such as kitchen and bedroom, while the hospital cockroaches with ESBL were found in the gutter 20 (20%) and in hospital environment 6 (6%).

The study of Salviati et al (2014) showed that ESBL producers *E.coli* were isolated from the environment of about 47.6 % from feces waste and boots swab, and 5.9% from the swab of swine farming. Bacteria resistance of the environment were spread through food and contaminated water and direct contact with the animals.

In Mesa's study (2006), ESBL producing bacterial were found in food sample like salad (tomato and lettuce), 0.4% in cooked food, and in waste exhaust was almost 100%. Aycan (2013) also reported that in hospital waste was found 89% *E.coli* that were ESBL producer. This study also found the combination of CTX-M+SHV gene. This was similar to the study by Dagi et al (2015) which found that 8% had CTX-M gene and 77.4% had the combination of TEM and CTX-M. This was because the plasmid coded CTX gene of plasmid IncFII plasmid type categorized into big plasmid. The common type of plasmid was F2:A-B-. This plasmid type was found in blaCTXM in Enterobacteriaceae isolated from other country. CTX-M gene found in other isolated were in plasmid which had high spreading ability (highly transmissible plasmids), so that the spreading was fast and efficient. The bacterial which expressed CTX-M was mostly co-resistant or multi resistant (Ramos et al. 2020).

The appearance and the wide spreading of ESBL among *E.coli* isolate clinic in the hospital became the main concern in some countries which human infected. This infection brought a great impact, because it could lead to the failure medication and the level of serious condition. ESBL consists of TEM, SHV, dan CTX-M. Among those, the number of highest variants was CTX-M. The existence of CTX-M made *E.coli* was resistant to any type of beta-lactam and transferred trough plasmid including unconnected microbes (Canton 2012).

The use of third generation of cephalosporin antibiotic, beta lactam type of antibiotic, and fluoroquinolone type of antibiotic in the hospital was suspected as the factor of the ESBL producer bacterial appearance. On the other hand, the utilization of antibiotic in the community also had a role in the spreading of resistant gene among bacterial species (Adelyap 2011). The higher incorrect antibiotic used, the higher evolution process selection and microorganism strain resistant proliferation (Pratiwi 2008).

In the last ten years, it was revealed that CTX-M almost changed other ESBL enzyme on Enterobacteriaceae, including TEM and SHV variants. The changes did not only happen as the result of the spreading of blaCTX-M gene on genetic incredible transfer mobilization including plasmid and transposon, but also the success of cloning (Rogers 2011). The increase of resistant phenomena which happened to the CTX-M producer organism to aminoglycoside, and fluoroquinolone also facilitated the selection process inn resistance.

Strength and limitation

The study is important public health concern, which is the potential transmission of antibiotic resistant bacteria by cockroaches in hospital and residential environments but, it only analyzes the presence of ESBL-producing Enterobacteriaceae in cockroaches and does not investigate the potential for transmission of these bacteria to humans or other organisms and the factors that contribute to the presence of ESBL-producing bacteria in cockroaches, such as the use of antibiotics in the environment or the presence of other potential vectors. This study uses a large sample size of 200

cockroaches, with 100 from each environment, which increases the validity and generalizability of the results and also, it combination of bacteriological culture and molecular methods to identify ESBL-producing bacteria,

CONCLUSION

The prevalence of ESBL producing bacteria among cockroach in hospital was 26 (26%), while in household cockroach was 14 (14%). It was significantly different among cockroaches in hospital and residential.

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Conflict of interest

None.

Funding disclosure

None.

Author contribution

CR and T[contributed to the conceptualization, the study design and methodology, and data collection. CR contributed to write and revise the manuscript. EB and M validation the data analysis, grammar and final content.

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