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Genetic Identification of bla_{CTX-M} Gene and bla_{TEM} Gene on Extended Spectrum Beta Lactamase (ESBL) Producing *Escherichia Coli* from Dogs

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Abstract

The purpose of this study was to identify the ESBL producing *E. coli* and the characteristics of the encoding genes. 84 rectal dog swabs from several animal clinics in Surabaya, Indonesia were taken for the study. All 84 positive *E. coli* samples were confirmed by the Double Disc Synergy Test (DDST) method. Molecular identification of bla_{CTX-M} and bla_{TEM} ESBL encoding genes using multiplex Polymerase Chain Reaction (PCR). ESBL-producing *E. coli* bacteria from rectal swabs were found to be 9.52% (8/84). The PCR results showed that the bla_{CTX-M} gene consisted of 8 ESBL isolates and bla_{TEM} gene as many as 1 ESBL isolate which have the potential as a reservoir in spreading antibiotic resistance to humans and animals.

Key words : *Escherichia coli*, ESBL, bla_{CTX-M} gene, bla_{TEM} gene, Dogs

Antibiotic resistance is a global threat which affects both the human and animal health by limiting the existence of antibiotic resistance,

in pet animals (Guerra *et al.*, 2014). *Escherichia coli* is a normal flora of the digestive tract of mammals that can cause diseases, in humans and animals (Dutta *et al.*, 2017) and act as a reservoir in the transmission of antibiotic resistance. It is helpful in tracking resistant genes that can spread from animals to humans and the environment (Hanhaboon *et al.*, 2015).

Extended Spectrum Beta Lactamase (ESBL) is an enzyme produced by gram negative bacteria Enterobacteriaceae, which can hydrolyze penicillin but also third generation cephalosporin and monobactam and other antibiotics (Pitout, 2012) by inactivation of penicillin and cephalosporin by using plasmid-mediated extended spectrum beta lactamase (ESBLs) such as the TEM-, SHV- or cefotaxime enzyme groups (CTX-M) (Bush, 2013).

Resistance caused by ESBLs is often associated with resistance to other group antibiotics commonly used in human medicine (WHO, 2014). Based on this background, efforts should be made to detect the ESBL encoding gene for *E. coli* isolated from dog rectal swabs which could

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Table 1. Details of primers used in this study

Primers	Sequences (5' to 3')	Target gene	Amplicons size	References
CTX-MA	CGC TTT GCG ATG TGC AG	bla _{CTX-M}	550-bp	Villegas <i>et al.</i> , 2004
CTX-MB	ACC GCG ATA TCG TTG GT			
TEM-F	ATA AAA TTC TTG AAG ACG AAA	bla _{TEM}	1086-bp	Yao <i>et al.</i> , 2007
TEM-R	GAC AGT TAC CAA TGC TTA ATC			

endanger public health.

Materials and Methods

84 rectal samples of dogs were taken from Central, Northern, Eastern, Southern and Western Surabaya from several animal clinics by sterile cotton swabs and put into 1% pepton water (E. Merck, Darmstadt, Germany), and the samples were immediately taken to the laboratory for examination (Safitri *et al.*, 2017).

Rectal swab samples were plated in Mac Conkey Agar (MCA) media (E. Merck, Darmstadt, Germany) and incubated at 37 ° C for 24 hours. Biochemical tests and gram staining (Krieg *et al.*, 2010) were performed. Furthermore, positive isolates of Escherichia coli were purified on Eosin Methylene Blue Agar (EMBA) media (E. Merck, Darmstadt, Germany) and incubated at 37 ° C for 24 hours (Effendi *et al.*, 2019).

Antibiotic resistance test on *E. coli* using the Double Disk Synergi Test (DST) was conducted using the standard Mac Farland 0.5 equivalent to 1.5 x 10⁸ CFU / ml (Effendi *et al.*, 2018). Using OXOID, Basingstoke, United

Kingdom: Interpretation of results by measuring the diameter of inhibition zone based on (CSLI, 2016).

Bacterial DNA extraction was grown overnight on agar media at 36°C and then colony was taken using 1µl sterile plastic loop and transferred to 1 ml sterile distilled water and added 5 µl lysozyme (5 mg / ml) and then incubated at 56°C for 30 minutes. The mixture was then centrifugated at 1000 rpm for 15 min at 4°C . The next step was to dilute DNA pellet into 100 µl with a buffer kit (QIAamp DNA mini kit 50). 1 µl of DNA was used for PCR amplification (Moenstein *et al.*, 2007).

Primer used to identify bla_{CTX-M} and bla_{TEM} genes. In the annealing process using 1 µl DNA solution Hot Star Taq Qiagen Master Mix and 10 pmol specific primers up to the final volume of 25 µl. Multiplex PCR amplification for the bla_{CTX-M} gene included 5 minute denaturation process at 95°C, 20 cycles of denaturation for 1 minute at 95°C, annealing 1 minute at 55°C, polymerization during 1 minute at 72°C

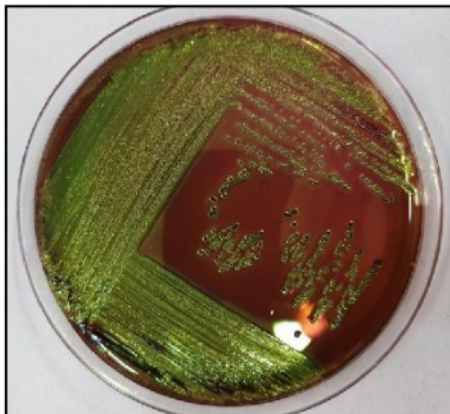


Fig 1. Macroscopic Escherichia coli colony on EMBA media



Fig 2. ESBL confirmation test using the DDST method (there appears to be an enlargement of the ESBL zone shown by the black arrow)

Table I. Data of ESBL isolates in this study

Location	Number of samples	Positive <i>E. coli</i>	ESBL Confirmation by DDST	bla _{CTX-M} gene	bla _{TEM} gene
Surabaya area	84	84	8	8	1

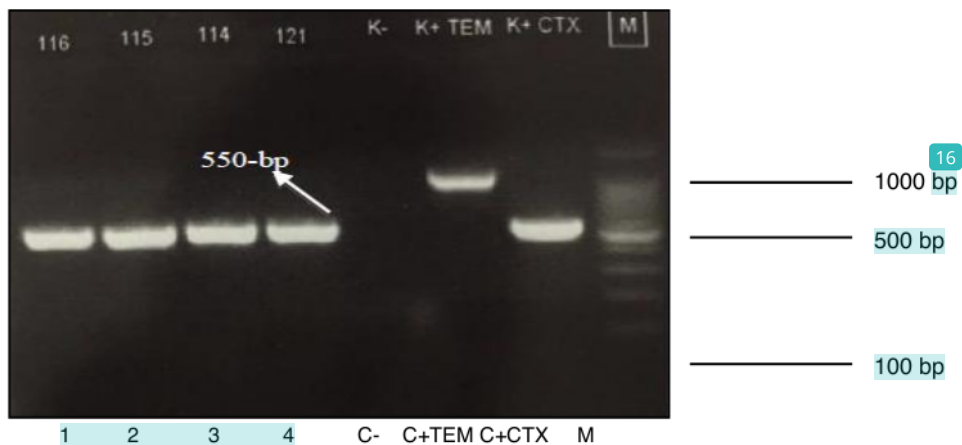


Fig 3. Electrophoresis results of four ESBL-producing *E. coli* isolates (bla_{CTX-M} at 550-bp) Lane 1-4 (ESBL isolates), C- (Non-ESBL), C+TEM (+ bla_{TEM}), C+CTX-M (+ bla_{CTX-M}), M (marker 100 bp)

and final extension for 20 minutes at 72°C with amplicons size 550-bp, and amplification for the bla_{TEM} gene with amplicons size 1086-bp (Villegas *et al.*, 2004). The next step is reacting the product PCR by electrophoresis with agarose gel 1% (w/v), which was then stained with etidium bromide and visualized using UV light.

Results and Discussion

The results of the isolation and identification of bacteria from 84 rectal dog swab samples were 84 samples (100%) positive for *E. coli*. The *E. coli* isolate was purified using Eosin Methylene Blue Agar (EMBA) media which appeared metallic green in color (Fig 1).

Confirmation of ESBL-producing *E. coli* using the Double Disc Synergy Test (DDST) method, which obtained eight positive ESBL samples (9.52%; 8/84) (Table I). In the DDST method there appears to be an enlargement of the ESBL zone with the synergistic pattern of the three antibiotics with clavulanate as its inhibitor (Fig 2).

The PCR results revealed 8 ESBL-producing *E. coli* isolates found in the bla_{CTX-M}

gene shown in the 550-bp band and 1 isolate (code of 118) in the Southern Surabaya area found bla_{CTX-M} 550-bp and bla_{TEM} genes in the band 1086-bp (Fig 4).

Isolation of ESBL-producing *Escherichia coli* among 84 rectal samples, were found in Surabaya region. Thus the companion animals can be a potential reservoir for the spread of antibiotic resistance to humans and the environment (Carvalho *et al.*, 2016).

In this study, the phenotypic and genotypic characteristics of the isolates tested confirmed the presence of the CTX-M enzyme, which causes resistance to cefotaxime (Bradford, 2001). The dominant bla_{CTX-M} gene was found at 100% (8/8) of ESBL isolates. The CTX-M enzyme has become the dominant ESBL in humans and it has been found normally that CTX-M encoding plasmids can also carry bla_{TEM} and other resistant genes such as aminoglycosides, chloramphenicol, sulfonamides, trimethoprim and tetracyclines (Ponnet, 2004).

The bla_{TEM} gene was found in one of eight ESBL-producing *E. coli* isolates (1/ 8)

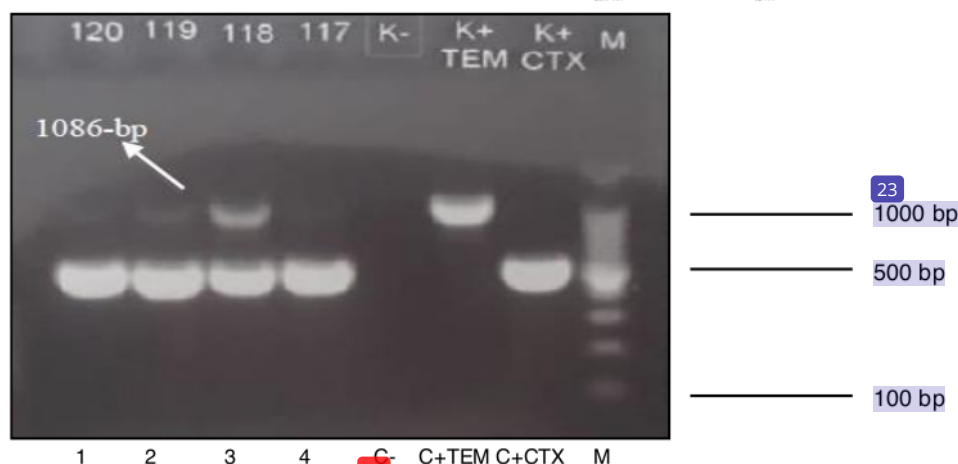


Fig 4. Electrophoresis results of four ESBL-producing *E. coli* isolates (4 of bla_{CTX-M} gene at 550-bp and 1 of bla_{TEM} gene at the 1086-bp band)

Lane 1-4 (ESBL isolates), C- (Non-ESBL), C+TEM (+ bla_{TEM}), C+CTX-M (+ bla_{CTX-M}), M (marker 100 bp)

code number 118 (Fig 4). Given that the bla_{CTX-M} and bla_{TEM} genes are the dominant gene type, which is consistently found in detecting resistance genes from companion animals in several countries (Huber *et al.*, 2013). Isolation of *E. coli* from cattle in China has increased rapidly in recent years with bla_{CTX-M} being the main gene coding that applies to ESBL (Rao *et al.*, 2014). It is known that, in general, ESBL genes are located on plasmids which can spread easily between commensal and pathogenic bacteria in flocks and the environment. However, bla_{TEM} gene producing *Klebsiella pneumoniae* from animals of food origin was dominant on ESBL producing *Klebsiella pneumoniae* (Effendi *et al.*, *loc. cit.*).

Efforts to prevent and control a case of ESBL occurrence in veterinary public health problem will be very easy if the source of transmission or the origin of the agent is known. It is difficult to predict the origin of enzymes, especially CTX-M, which is predominantly found in the environment, due to differences in amino acids between subgroups (between 70 to 90% sequences) and geographical differences from host strains are also very influential (Bonnet *et al.*, 2001). Efforts must be made to prevent and control it by regularly testing the susceptibility to cefotaxime, ceftriaxone or cefepim and ceftazidime (Quinteros *et al.*, 2003).

Summary

Genetic identification showed the dominance of the bla_{CTX-M} gene compared to the bla_{TEM} gene, and these results showed that ESBL producing *Escherichia coli* from p₉ dogs have relatively high results. Therefore, ESBL producing *E. coli* showed the potential for spreading and poses a threat to animal health and public health from the bla_{CTX-M} gene of *E. coli* isolates.

Acknowledgement

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References

- Bonnet, R., C. Dutour, J.L.M., Sampaio, C., Chanal, D., Sirot, R., Labia, C., De Champs, and J. Sirot. (2001) Novel cefotaximase (CTX-M-16) with increased catalytic efficiency due to substitution Asp-2403 Gly. *Antimicrob. Agents Chemother.* **45**:2269–2275.
- Bonnet, R. (2004) Growing group of extended spectrum beta lactamases: the CTX-M enzymes. *Antimicrob. Agents Chemother.* **48**: 1-14.
- Bradford, P.A. (2001) Extended spectrum beta lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. *Clin Microbiol Rev.* **14**: 933-951.
- Bush, K. (2013) Proliferation and significance of clinically relevant β -lactamases. *Ann N Y Acad Sci.* **1277**:84–90.
- Carvalho, A.C., Barbosa, A.V., Araisp, L.R., Ribeiro, P.F.,

- Carneiro, V.C., and Cerqueira, A.M.F. (2016) Resistance patterns, ESBL genes, and genetic relatedness of *Escherichia coli* from dogs and owners. *Brazilian Journal of Microbiol.* **47**:150-158.
- Dutta, A., M.S. Jalal, S.K. Nath, P.K. Dhar, A. Das and M.M. Uddin. (2017) Multi drug resistance pattern of *Escherichia coli* isolated from hospital effluent and determination of tetracycline resistance gene. *J. Inf. Mol. Biol.* **4**(3): 49-53.
- Effendi, M.H., Bintari, I.G., Aksono, E.B., and Hermawan, I.P. (2018) Detection of *bla*_{TEM} Gene of *Klebsiella pneumoniae* Isolated from Swab of Food-Producing Animals in East Java. *Tropical Animal Science Journal.* **41**(3):174-178
- Effendi, M.H., Harijani, N., Budiarto, Triningtya, N.P., Tyasningsih, W. and Plumeriastuti, H. (2019) Prevalence of Pathogenic *Escherichia Coli* Isolated from Subclinical Mastitis in East Java Province, Indonesia. *Indian Vet. J.* **96** (03) : 22 - 25
- Hanhaboon, P., Kimprasit, T., Wajjwalku, W. and Amavisit, P. (2015) Extended Spectrum β -lactamase Producing *Escherichia coli* isolated from infected canines. *Thai. J. Vet. Med.* **45**(2): 263-267.
- Huber, H., Zweifel, C., Wittenbrink, M.M., and Stephan, R. (2013) ESBL-producing uropathogenic *Escherichia coli* isolated from dogs and cats in Switzerland. *Vet Microbiol.* **162** (2-4): 992-996.
- Krieg, N.R., Staley, J.T., Brown, D.R., Hedlund, B.P., Paster, B.J., Ward, N.L., Ludwig, W. and William B. and Whitman., W.B. (2010) *BERGEY'S MANUAL OF Systematic Bacteriology*. Second Edition, Volume Four, Springer New York, Dordrecht, Heidelberg, London, pp 823-834.
- Monstein, H.J., Ostholm-Bulkhed, A., Nilsson, M.V., M. Dombusch., K. and Nilsson, L.E. (2007) Multiplex PCR amplification assay for the detection of *bla*SHV, *bla*TEM, and *bla*CTX-M genes in Enterobacteriaceae. *APMIS.* **115**(12): 1400-1408.
- Quinteros, M., Radice, M., Gardella, N.M., Rodríguez, N. Costa, D. Korbenfeld, E. Couto, G. and Microbiology Study Group. (2003) Extended-spectrum β -lactamases in *Enterobacteriaceae* in Buenos Aires, Argentina, public hospitals. *Antimicrob. Agents Chemother.* **47**:2864–2867.
- Rao, L, Lv, L, Zeng, Z., Chen, S., He, D., Chen, X., Wu, C., Wang, Y., Yang, T., Wu, P., Liu, Y., and Liu, J.H. (2014) Increasing prevalence of extended-spectrum cephalosporin-resistant *Escherichia coli* in food animals and the diversity of CTX-M genotypes during 2003-2012. *Vet. Microbiol.* **172**:534–541.
- Safitri, R.D., Cicilia, R., Bintari, I. G., Hermawan, I.P., Effendi, M.H., Ernawati, R., and Rahmahani. J. (2017) Detection Of Encoding Gene Extended Spectrum Beta Lactamase on *Escherichia coli* Isolated From Broiler Chicken Meat In Traditional Market Surabaya. *International J. of Develop. Research.* **07** (11): 17354-17357
- Villegas, M. V., Correa, A., Perez, F., Zuluaga, T., Radice, M., and Gutkind, G. (2004) CTX-M-12 beta-lactamase in a *Klebsiella pneumoniae* clinical isolate in Colombia. *Antimicrob. Agents Chemother.* **48**: 629–631.
- World Health Organization (WHO). (2014) Antimicrobial resistance: global report on surveillance. World Health Organization, pp 5-17.

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Publication

13 aip.scitation.org
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14 biotechnologyforbiofuels.biomedcentral.com
Internet Source

15 www.pjms.com.pk
Internet Source

16 d-nb.info
Internet Source

17 Jan Walther-Rasmussen. "Cefotaximases (CTX-M-ases), an expanding family of extended-spectrum β -lactamases", Canadian Journal of Microbiology, 03/2004
Publication

18 P. L. Ho. "Detection and characterization of extended-spectrum β -lactamases among bloodstream isolates of Enterobacter spp. in Hong Kong, 2000-2002", Journal of Antimicrobial Chemotherapy, 01/19/2005
Publication

19 Randall S. Singer, Sheila K. Patterson, Richard L. Wallace. " Effects of Therapeutic Ceftiofur Administration to Dairy Cattle on Dynamics in the Intestinal Tract ", Applied and Environmental Microbiology, 2008
Publication

20 Xiaoqiang Liu, Xueqi Wei, Li Liu, Xiaolan Feng, Zhengqi Shao, Zilong Han, Yinqian Li. "Prevalence and characteristics of extended-spectrum β -lactamases-producing *Escherichia coli* from broiler chickens at different day-age", Poultry Science, 2020
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21 aac.asm.org
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22 journal.ugm.ac.id
Internet Source

23 mafiadoc.com
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24 Eddy SUKMAWINATA, Wataru SATO, Shuya MITOMA, Takuya KANDA et al. "Extended-spectrum β -lactamase-producing *Escherichia coli* isolated from healthy Thoroughbred racehorses in Japan", Journal of Equine Science, 2019
Publication

25

Feng YANG, Shi-dong ZHANG, Xiao-fei SHANG, Xu-rong WANG, Ling WANG, Zuo-ting YAN, Hong-sheng LI. "Prevalence and characteristics of extended spectrum β -lactamase-producing Escherichia coli from bovine mastitis cases in China", Journal of Integrative Agriculture, 2018

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