VOL 97 No. 01 JANUARY, 2020

Print ISSN 0019 - 6479
E - ISSN 0974 - 9365

₹. 80

THE INDIAN VETERINARY JOURNAL

SINCE - 1924

Journal of the

INDIAN VETERINARY ASSOCIATION

ESTD - 1922

Regd. No. Sl. No. 96/1967 (CHENNAI)

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Vol. 97 JANUARY 2020 No. 01

THE INDIAN VETERINARY JOURNAL

(Official Organ of the Indian Veterinary Association)

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Vol. 97 January 2020 No. 01

CONTENTS

CLINICAL AND FIELD ARTICLES :

and Identification of Aerobic Bacteria Detected From Sheep Infected With Pneumonia. *Adv. Environ. Biol.* **10(5)**: 214-219.

Munoz MA, Welcome FL, Schukken YH and Zadoks RN (2007) Molecular epidemiology of two Klebsiella pneumoniae mastitis outbreaks on a dairy farm in New York State. *J Clin Microbiol*. **45**: 3964–3971

Ohnishi M, Okatani AT, Harada K, Sawada T and Marumo K (2013) Genetic Characteristics of CTX-M-Type Extended-Spectrum-b-Lactamase (ESBL)-Producing Enterobacteriaceae Involved in Mastitis Cases on Japanese Dairy Farms, 2007 to 2011. *J Clin Microbiol.* **51**: 3117–3122.

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Indian Vet. J., January 2020, 97 (01) : 17 - 21

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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(Received : May, 2019 **152/19** Accepted : September, 2019)

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 the 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 mutiplex 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 I. 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 *et al*., 2004
CTX-MB	ACC GCG ATA TCG TTG GT			
TEM-F	ATA AAA TTC TTG AAG ACG AAA	bla_{TEM}	1086-bp	Yao *et al.,* 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 (DDST) was conducted using the standard Mac Farland 0.5 equivalent to 1.5 x 10^8 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. Mutiplex PCR amplification for the bla_{CTX-M} gene included a 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

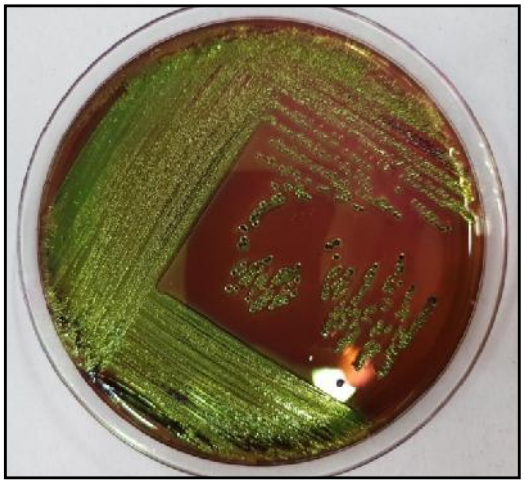

Fig 1. Macroscopic Escherichia coli colony on EMBA media

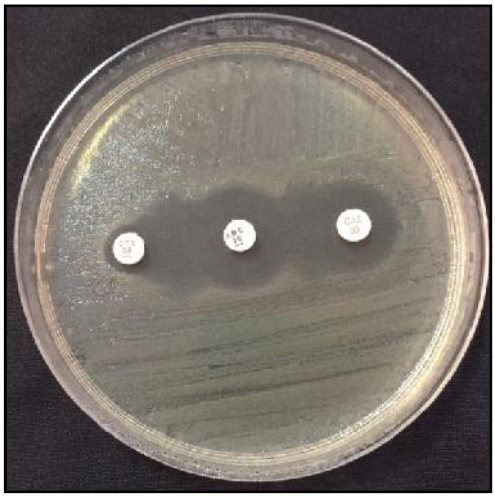

Fig 2. ESBL confirmation test using the DDST method (there appears to be an enlargement of the ESBL zone shown by the black arrow)

Table I. Data of ESBL isolates in this study

Location	Number of samples	Positive *E. coli*	ESBL Confimation by DDST	bla_{CTX-M} gene	bla_{TEM} gene
Surabaya area	84	84	8	8	1

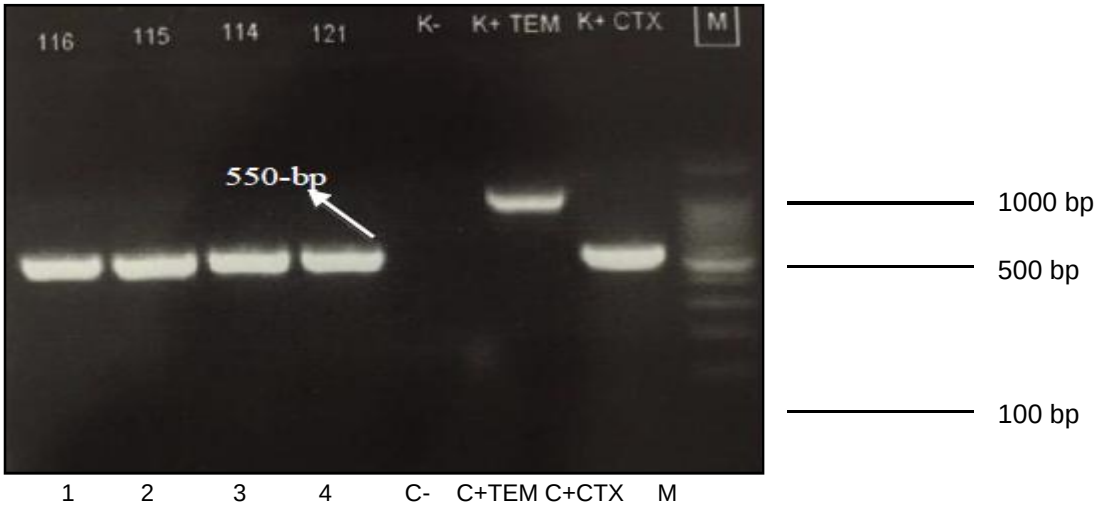

Fig 3. Electrophoresis results of four ESBL-producing E. coli isolates (bla_{CTX-M} at 550-bp)
Lane 1-4 (ESBL isolates), C- (Non-ESBL), C+TEM (+ bla_{TEM}), C+CTX-M (+ bla_{CTX-M}), M (marker 100 bp)

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). 1 2 3 4 C- C+TEM C+CTX M

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, trimethropim and tetracyclines (Bonnet, 2004).

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

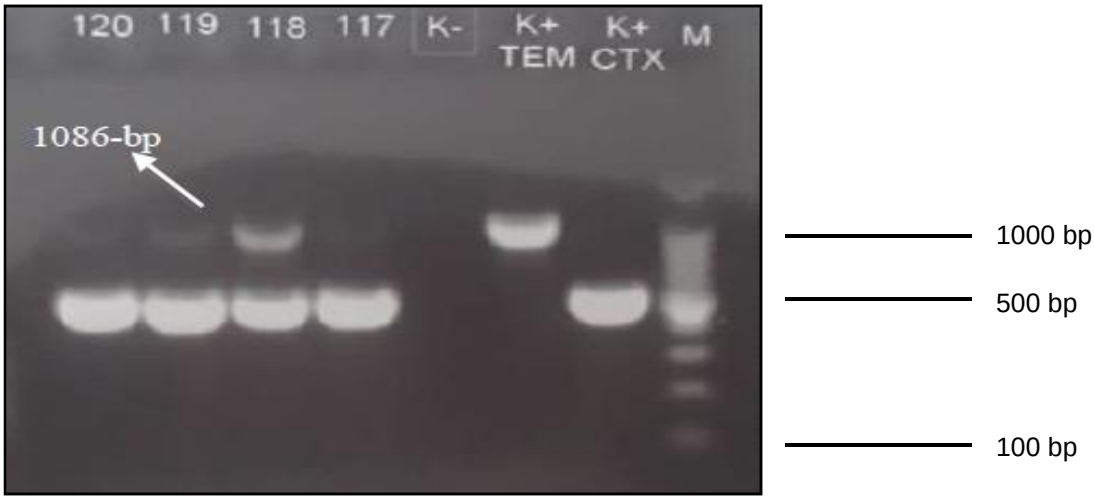

Fig 4. Electrophoresis results of four ESBL-producing E. coli isolates (4 of bla$_{CTX-M}$ gene at 550-bp and 1 of bla$_{TEM}$ gene at the 1086-bp band)
Lane 1-4 (ESBL isolates), C- (Non-ESBL), C+TEM (+ bla$_{TEM}$), C+CTX-M (+ bla$_{CTX-M}$), M (marker 100 bp)

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 commonsal 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 occurence 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 pet 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

This study was supported in part with the Hibah Mandat Fund from Airlangga University, Indonesia.

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Indian Vet. J., January 2020, 97 (01) : 21 - 23

Ethno Veterinary Practices Followed by Farmers for Goats in Pachaimalai Hills of Tamilnadu

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(Received : June, 2019 **230/19** Accepted : July, 2019)

Abstract

Survey was conducted during 2017 – 2018 to document the herbal medicinal practices prevalent in the Perambalur and Tiruchirappalli district in the Pachaimalai hills region of TamilNadu. Field survey in 8 villages in the Perambalur and Tiruchirappalli district was carried out involving 200 respondents through participatory rural appraisal. A total of 21 species of herbal plants is used either alone or in combination as traditional remedies for 14 different disorders/diseases in goats.

Key words : Ethnoveterinary, Pachaimalai hills, Goats.

Ethno veterinary practices provide

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