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## Molecular Identification of Extended Spectrum Beta-Lactamase (ESBL) Producing *Escherichia coli* Isolated From Dairy Cows in East Java Province, Indonesia

Akyun Rozaqi Syah Putra, Mustofa Helmi Effendi<sup>1</sup>, Setiawan Koesdarto and Wiwiek Tyasningsih

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### Abstract

Occurrence of extended spectrum beta-lactamases (ESBL) in *Escherichia coli* (*E. coli*) from animal origin is a growing health concern of global significance. The objective of this study was to determine the occurrence of ESBL producing *E. coli* and the characteristics of their encoding genes from 115 rectal swab samples of dairy cows from Tululungagung and Surabaya farms. All samples were positive for *Escherichia coli* as per indole test. To confirm the ESBL the Double Disc Synergy Test (DDST). Betalactam antibiotic disk namely Amoxiclyln-clavulanate, Ceftazidime and Cefotaxime were used for DDST. Molecular identification for bla<sub>CTX-M</sub> and bla<sub>TEM</sub> ESBL encoding genes was done by used Polymerase Chain Reaction. The *Escherichia coli* bacteria isolated from rectal swabs of dairy cows was 5.21% (6/115). PCR results showed that bla<sub>CTX-M</sub> gene was 6 ESBL isolates and bla<sub>TEM</sub> gene was 2 ESBL isolates. It can be concluded that dairy cows can be potential as reservoir for spreading ESBL isolates to human health.

**Key words:** *Escherichia coli*, ESBL, DDST, bla<sub>CTX-M</sub> gene, bla<sub>TEM</sub> gene, PCR

Producing bacteria is a major threat to public health. The existence of this Extended-Spectrum Beta-Lactamases (ESBLs) is due to an improper use of antibiotics in infections and treatment failure, (Effendi *et al.*, 2018).

ESBLs producing bacteria which induces resistance to the expanded spectrum of cephalosporins and monobaktam but does not affect cephamycins or carbapenem and is inhibited by beta-lactamase inhibitors such as clavulanate, sulbactam, and tazobactam (Peterson and

Bonomo, 2005). Widespread third generation cephalosporins and aztreonam are believed to be the main cause for the mutations, which have led to the emergence of ESBL (Al-Jasser, 2006; Bradford, 2001).

The prevalence of ESBL-producing *E. coli* in food-producing animals and food products is very high. The study was to detect the presence of ESBLs-producing bacteria from dairy cows by double discs synergy test (DDST) and phenotypic confirmation test to detect ESBL producing bacteria and to identify encoding genes for ESBL producing *E. coli*.

### Materials and Methods

During the period July 2018 to March 2019, a total of 115 samples from rectal swabs from dairy cows (50 samples from Tulungagung dairy farm and 65 samples from Surabaya dairy farm) were collected, using a sterile swab inserted into the eppendorf tube containing the Pepton water buffer media (Safitri *et al.*, 2017). Samples were taken to the laboratory in a thermobox container at 4°C.

Each swab sample was inoculated on Brilliant Green Bile Broth (BGBB) media and then incubated at 37°C for 24 hours. The positive results are characterized by the presence of gas bubbles in the Durham tube and the change of green colour to cloudy green. They were grown on Eosin Methylene Blue Agar (EMBA) media by streaking and incubated at 37°C for 18-24 hours. Typical *E. coli* colonies on EMBA media was metallic green, and it was planted again in Pepton Water and incubated at 37°C for 24 hours. The incubated Pepton Water media is dripped with Kovach reagents in two or three drops. A positive *E. coli* test is characterized by

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**Table I.** Data of ESBL isolates in this study

Location	Number of samples	Positive <i>E. coli</i>	ESBL Confirmation by DDST	<i>bla</i> <sub>CTX-M</sub> gene	<i>bla</i> <sub>TEM</sub> gene
Tulungagung farm	50	50	3	3	1
Surabaya farm	65	65	3	3	1
<b>Total</b>	<b>115</b>	<b>115</b>	<b>6</b>	<b>6</b>	<b>2</b>

the formation of a red ring on the surface of the Pepton Water media (Effendi *et al.*, 2019).

Confirmation test for ESBL producing *E. coli* by using disk antibiotic (OXOID, Basingstoke, United Kingdom) amoxicillin-clavulanate 30 µg (CT0223B), Cefotaxime 30 µg (CT0166), Ceftazidime 30 µg (CT0412), as per Clinical and Laboratory Standards Institutions (CLSI, 2016).

A total of 115 positive samples of *E. coli* were tested with Double disc synergy test (DDST) to detect synergy between a disc of augmentin (Amoxicillin and clavulanic acid) and third generation cephalosporins. The clavulanate in augmentin disc diffuses through the agar and inhibits the beta-lactamases, surrounding third generation cephalosporin disc. Discs containing 30µg of ceftazidime, and cefotaxime were placed over inoculated Mueller-Hinton agar plates 20 mm apart from centrally placed amoxicillin-clavulanic acid disc (20/10 µg). Following overnight incubation at 37°C, diameter of zone of inhibition was measured.

The initial step of PCR DNA extraction of bacterial culture in EMBA media was made as per (Yanestria *et al.*, 2019) and tested by specific primers for the *bla*<sub>TEM</sub> and *bla*<sub>CTX-M</sub> genes as described Ali *et al.*, (2016) with slight modifications in cycling conditions, Table I. *Taq* DNA polymerase enzyme, deoxyribonucleotide triphosphates and buffers used in the PCR mixture were obtained from Thermo Fisher Scientific Inc. (Massachusetts, USA). Thermo-cycling reaction was conducted for denaturation at 94°C for 2 minutes, extended denaturation at 94°C for 1 minutes, annealing for 52°C for 30 sec, extended at 72°C at 45 sec, and extended at 72°C for 5 minutes, this reaction is 30 cycles. PCR product was visualized in mini gel electrophoresis and documented in UV Reader/Gel Documentation System.

## Results and Discussion

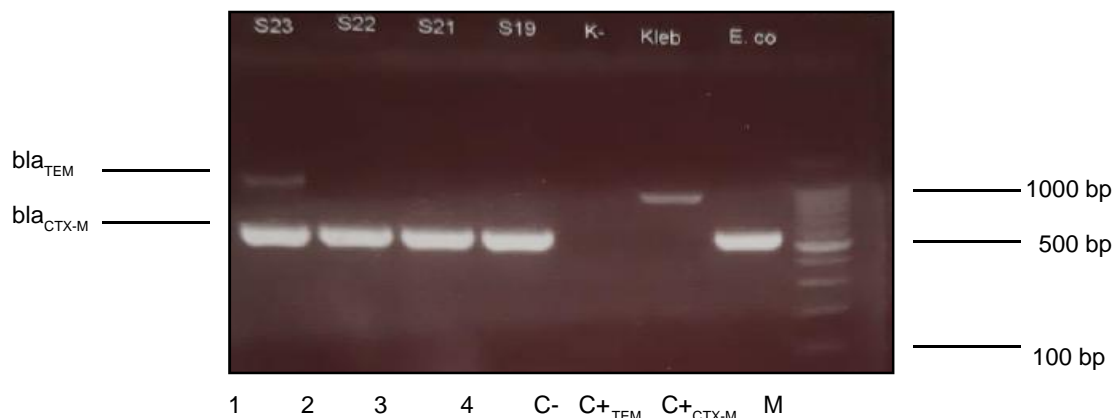
The isolation and identification results of 115 samples showed the changes in green colour to cloudy green is presented in table I. All the 115 samples were positive for *E. coli* (100%). These results are in accordance with the finding of Rasheed *et al.* (2014) who reported that the level of ESBL producing *E. coli* contamination in cattle was found to be 3.3%, however the study conducted by Wasinki *et al.* (2013) reported that the level of *E. coli* contamination was 13.5% in food animals. Enterobacteriaceae bacteria in faecal samples can cause carcass contamination and as a potential for pollution in meat products (Geser *et al.*, 2011).

Extension of the edge of the inhibition zone of ceftazidime, and cefotaxime disc on the side exposed to the disc containing amoxicillin-clavulanic acid was positive for ESBL. DDST showed that ESBL producing *E. coli* were 6 (5.21%) isolates (Table I). The test image results of the DDST is shown in Fig 1.

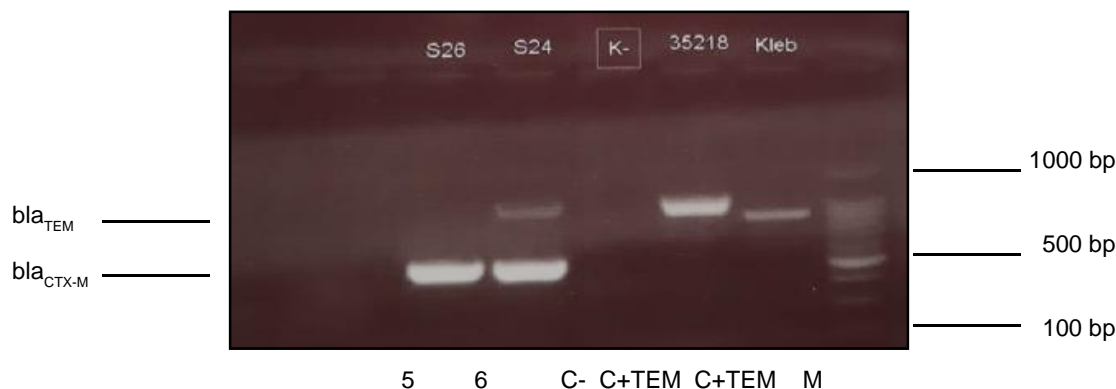
The prevalence of bacteria. ESBL-producing *coli* and their evolution are due to

**Fig 1.** Double Disc Synergy Test (DDST) for ESBL confirmation

Molecular Identification of Extended ...



**Fig 2.** PCR profiles of molecular detection bla<sub>CTX-M</sub> gene and bla<sub>TEM</sub> gene of ESBLs  
Lane 1-4 (ESBL isolates), C- (Non-ESBLs), C+TEM (+ bla<sub>TEM</sub>), C+CTX-M (+ bla<sub>CTX-M</sub>), M (marker 100 bp)



**Fig 3.** PCR profiles of molecular detection bla<sub>CTX-M</sub> gene and bla<sub>TEM</sub> gene of ESBLs  
Lane 5-6 (ESBL isolates), C- (Non-ESBLs), C+TEM (+ bla<sub>TEM</sub>), M (marker 100 bp)

the frequent administration of drugs such as penicillin, cephalosporin, monobactam, and carbapenem (Cheaito and Matar, 2014), which is associated with resistance to other types of antibiotics leading to Multidrug resistance. Haldorsen (2011) who has reported that genes coding for resistance enzymes such as AME (Aminoglycoside modifying enzyme) and ESBL are often found in bacterial plasmids. One of the main causes of the increasing prevalence of bacteria that are resistant to both beta-lactam and aminoglycoside group antibiotics is the presence of gene transfer that occurs in plasmid, integron, and transposon (Halderson *loc. cit*; Allocati *et al.*, 2013). Furthermore, the combination of several resistant genes causes bacteria to be resistant to most classes of antibiotics (Allocati *et al.*, *loc. cit*).

Our findings from molecular identifica-

tion illustrate that bla<sub>CTX-M</sub> gene is the most common genotype, followed by bla<sub>TEM</sub> gene (Table I) revealed on Figure 2 and 3. This finding is similar to in Turkey (Tekiner & Özpınar, 2016) and other studies from China (Liu *et al.*, 2015) and around the world that also report bla<sub>CTX-M</sub> as the dominant ESBL genotype (Geser *et al.*, 2012; Kar *et al.*, 2015). This is in line with recent detection of bla<sub>CTX-M</sub> produced by *E. coli* from cattle and other food animals in Egypt (Braun *et al.*, 2016), East Asia (Yu *et al.*, 2015), India (Upadhyay *et al.*, 2015), United Kingdom (Timofte *et al.*, 2014), and Tanzania (Seni *et al.*, 2016). Similarly, in animals, the prevalence of ESBL-producing *E. coli* in China has increased rapidly in years with bla<sub>CTX-M</sub> 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.

### Summary

There is a high incidence of ESBL-producing *E. coli* in dairy cows. Molecular identification showed the dominance of the bla<sub>CTX-M</sub> gene compared to the bla<sub>TEM</sub> gene. The ESBL producing *E. coli* showed the potential for rapid and wider dissemination and poses a threat to animal health and public health. There is an alarming high prevalence of bla<sub>CTX-M</sub> in ESBL producing *E. coli* from dairy cows in Tulungagung and Surabaya farms in East Java Province, Indonesia.

### Acknowledgement

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## Acupuncture Could Increase Spermatogenic Cells in Albino Rats Exposed to Heat Stroke

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### Abstract

This study used 20 albino rats divided into five treatments i.e., negative control group (C-) not exposed to heat stroke and acupuncture and positive control group (C+) exposed to heat stroke. Treatment groups (T1, T2, T3) were exposed to heat stroke and for 5; 10; 20 seconds/day followed by acupuncture. The testis sections were prepared with hematoxylin-eosin staining and the blood serum was collected to assay testosterone by ELISA kit. The result of spermatogenic cells showed significant difference ( $p < 0.05$ ) on treatment group (T3), but the result of testosterone levels was not significantly different ( $p > 0.05$ ) among all treatments.

**Key words:** Acupuncture, Heat stroke, Spermatogenic cells, rats.

Global warming is a worldwide problem that increases the risk of heat stroke. Heat stroke is an extreme temperature environment that can cause infertility (Leon and Helwig,

2010). Acupuncture has been developed as a traditional medical technique (Siterman *et al.*, 2009). The aim of this study was to examine the potential of acupuncture on spermatogenic cells and testosterone levels in albino rats exposed to heat stroke.

### Materials and Methods

This study was approved by ethical committee Faculty of Veterinary Medicine, Universitas Airlangga with registration number No.686-KE. This study used 20 albino rats that were adapted for a week and divided into five groups i.e., negative control group (C-) not exposed to heat stroke and acupuncture and positive control group (C+) exposed to heat stroke. Treatment groups (T1, T2, T3) were exposed to heat stroke and for 5; 10; 20 seconds/day followed by acupuncture.

Incubator was set at 45°C for 20 seconds to conduct a heat stroke effect. Acupuncture was done for a week using a specific needle for meridian point, targeted as a reproductive

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