



Contents lists available at [ScienceDirect](http://www.elsevier.com/locate/apjtb)

Asian Pacific Journal of Tropical Biomedicine

journal homepage: www.elsevier.com/locate/apjtb



Original article <http://dx.doi.org/10.1016/j.apjtb.2017.07.012>

Multidrug resistance extended spectrum β -lactamase and AmpC producing *Escherichia coli* isolated from the environment of Bogor Slaughterhouse, Indonesia



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ARTICLE INFO

Article history:

Received 8 Oct 2016

Received in revised form 28 Nov 2016

Accepted 26 Jul 2017

Available online 1 Aug 2017

Keywords:

Escherichia coli

Environment

Extended spectrum β -lactamase

Slaughterhouse

ABSTRACT

Objective: To determine the multidrug resistance extended spectrum β -lactamase and AmpC (ESBL/AmpC producing) *Escherichia coli* (*E. coli*) isolated from the environment of Bogor slaughterhouse, Indonesia.

Methods: A total of 35 samples from 7 locations in slaughterhouse *i.e.*, source of water, slaughtering floor, swab of carcass area floor, swab of evisceration area floor, untreated waste water, treated waste water, drinking water for cattle were collected from March to April 2016. Presence of ESBL/AmpC producing *E. coli* and susceptibility testing against 8 antimicrobial agents (penicillin G, streptomycin, gentamycin, ciprofloxacin, enrofloxacin, tetracycline, trimethoprim-sulfamethoxazole, and polymyxin B) were detected by disk diffusion test according to Clinical and Laboratory Standards Institute.

Results: ESBL/AmpC producing *E. coli* were identified in 14.3% (5/35) of the collected samples from the environment of Bogor slaughterhouse. ESBL/AmpC-producing *E. coli* isolates were detected in untreated waste water ($n = 3$), slaughtering floor ($n = 1$), and carcass area floor ($n = 1$). Most of ESBL/AmpC-producing *E. coli* isolates (80%) showed multidrug resistance phenotypes against at least three classes of antibiotics. The highest incidence of antibiotics resistance was against penicillin G (100.0%) and streptomycin (100.0%), followed by gentamicin (60.0%), trimethoprim-sulfamethoxazole (60.0%), tetracycline (40.0%), ciprofloxacin (40.0%), enrofloxacin (20.0%), and polymyxin B (0.0%).

Conclusions: The transmission of antimicrobial resistant bacteria into the environment may be a potential risk for human health.

1. Introduction

Extended spectrum β -lactamase and AmpC producing (ESBL/AmpC producing) Enterobacteriaceae have been known distributed in humans, animal, foods of animal origin and environment [1,2]. These enzymes have ability to inactivate the third

generation cephalosporin, by hydrolyzing their β -lactam ring [3]. The increasing of mutations of ESBL gene becomes an emerging infection on human health and food production of animal origin [4]. ESBL/AmpC genes are often located on mobile genetic plasmids which can be easily transmitted within and between different bacterial species and hosts [5]. Increasing number of ESBL/AmpC producing *Escherichia coli* (*E. coli*) in foods produced by animal showed that animals might become source of contamination of the animal products [6]. Additionally, plasmid mediated AmpC and carbapenemases are known to become a potential risk in human and veterinary medicine [3].

The occurrence of 8.6% of ESBL producing *E. coli* isolated from cattle feces has been reported in Bogor Slaughterhouse at previous study. Fecal contamination into the environment, such as surface water could be the potential transmission for

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Foundation project: The research was support by Budget Implementation Registration Form of Bogor Agricultural University with number: 079/SP2H/LT/DRPM/II/2016.

Peer review under responsibility of Hainan Medical University. The journal implements double-blind peer review practiced by specially invited international editorial board members.

infectious diseases. ESBL producing *E. coli* showed that all isolates exhibited multidrug (MDR) resistance phenotypes to at least four antibiotics [7]. Multidrug resistance Enterobacteriaceae has been reported as the global issues of treatment problem in human and animals [8]. There has not been much research regarding the presences of ESBL producing *E. coli*, particularly from the environment, in Indonesia. For this reason, this study was to determine the MDR ESBL/AmpC producing *Escherichia coli* isolated from the environment of Bogor slaughterhouse, Indonesia.

2. Material and methods

2.1. Screening test of ESBL/AmpC producing *Escherichia coli*

Screening test of ESBL/AmpC producing *E. coli* was done by referring to Sudarwanto *et al.* [7]. A total of 35 samples from 7 locations in slaughterhouse *i.e.*, source of water, slaughtering floor, swab of carcass area floor, swab of evisceration area floor, untreated waste water, treated waste water, drinking water for cattle in the environment of Bogor slaughterhouse were collected from March to April 2016. Sampling was done with five times repetition for each location on different days. The samples were put in sterile plastic bags and transported to the laboratory using cooling box. Homogenization was done by mixing sample of 0.1% buffered peptone water (BPW; Oxoid CM1049, England) as much as 1: 9. For the enrichment, rinsates (10 mL) were supplemented with 20 µL cefotaxime (1 µg/mL) and incubated for 24 h at 37 °C. There after, the enrichment was inoculated onto MacConkay agar (Merck 1.05465.0500, Germany) supplemented with 1 mg/L cefotaxime, and incubated at 37 °C for 24 h under aerobic condition. The colonies which presumed as *E. coli* will appear as red colonies in the media and surrounded by turbid zone. Further works were continued by KOH test, Gram staining, oxidase test (Oxoid MB0266A, England), sulfide, indole, and

2.2. Phenotypic confirmation test of ESBL/AmpC and antibiotic susceptibility testing

All isolates that showed cefotaxime resistant and KOH-positive were confirmed for ESBL production by the disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI) guidelines [9]. Antibiotic disks that were used to evaluated production of ESBL/AmpC are 10 µg of cefpodoxime, 10 µg of cefpodoxime combination with ESBL inhibitor, 10 µg of cefpodoxime combination with AmpC inhibitor, and 10 mg of cefpodoxime combination with ESBL and AmpC inhibitor (MAST Group, Germany). *E. coli* isolates which produced ESBL/AmpC were determined for antibiotic resistant against 8 antimicrobial agents (penicillin G, streptomycin, gentamycin, ciprofloxacin, enrofloxacin, tetracycline, trimethoprim-sulfamethoxazole, and polymyxin B) with disk diffusion method according to CLSI protocols and evaluated with CLSI criteria. *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as a control strain.

3. Results

Overall, ESBL/AmpC producing *E. coli* could be identified in 14.3% (5/35) of the collected samples from the environment of Bogor slaughterhouse. ESBL/AmpC-producing *E. coli* isolates were detected in untreated waste water ($n = 3$), slaughtering area floor ($n = 1$), and swab of carcass area floor ($n = 1$). Most of ESBL/AmpC-producing *E. coli* isolates (80%) showed multidrug resistance phenotypes against at least three classes of antibiotics. The highest incidence of antibiotics resistance was against penicillin G (100.0%) and streptomycin (100.0%), followed by gentamicin (60.0%), trimethoprim-sulfamethoxazole (60.0%), tetracycline (40.0%), ciprofloxacin (40.0%), enrofloxacin (20.0%), and polymyxin B (0.0%). Detail results on characteristics and antibiotic susceptibilities of multidrug resistant of 5 ESBL/AmpC-producing *E. coli* isolates were described in Table 1.

Table 1

Multidrug resistance ESBL/AmpC-producing *E. coli* in the environment of Bogor slaughterhouse.

Sample Code	Source of ESBL/AmpC-positive samples	Antibiotic resistance ^a												
		β-lactam			Aminoglycosides		Fluoroquinolones		Tetracycline	Folic acid inhibitor	Lipopeptide	Total ^b		
		P	S	CN	CIP	ENR	TE	STX	PB	R	I	S		
4b/SSB	Untreated waste water	R	R	R	R	R	R	R	R	S	7	0	1	
3/SSB	Untreated waste water	R	R	R	R	I	I	S	R	S	5	1	2	
5/SSB	Untreated waste water	R	R	S	I	I	S	R	S	S	3	3	2	
5/LP	Slaughtering area floor	R	R	R	S	I	S	S	S	S	3	1	4	
1/LK	Swab of carcass area floor	R	R	S	I	I	R	S	S	S	3	3	2	

^a P: penicillin G; S: streptomycin; CN: gentamicin; CIP: ciprofloxacin; ENR: enrofloxacin; TE: tetracycline; STX: trimethoprim-sulfamethoxazole; PB: polymyxin B.

^b R: resistance; I: Intermediate; S: sensitive.

motility (SIM) test, and biochemical test (indole, methyl red, Voges-Proskauer, and citrate [IMViC]). The suspected pink colony isolates were sub cultured onto tryptic soy broth (Merck 1.05458.0500, Germany) and incubated at 37 °C for 24 h. The identification of the *E. coli*-like colonies was then confirmed using Analytical Profile Index 20E (Biomérieux, United States).

4. Discussion

This is the first study of MDR ESBL/AmpC-producing *E. coli* in the environment of Bogor slaughterhouse, Indonesia. ESBL/AmpC-producing *E. coli* isolates were detected in 5 of the 35 (6.0%) samples. Based on the sampling location, ESBL/

AmpC-producing *E. coli* isolates were detected in untreated waste water 60% (3/5), slaughtering area floor 20% (1/5), and swab of carcass area floor 20% (1/5).

Several study reported that the prevalence of ESBL/AmpC producing *E. coli* is increasing during the last decade [10]. These bacteria could be easily transmitted to the environment by fecal contamination [11]. Nüesch-Inderbinen and Stephan [12] stated that ESBL/AmpC producing commensal *E. coli* could be transmitted from the environment of livestock, including air, soil, and fecal contamination to humans around the farms. Previous study has also demonstrated CTX-M producing *Escherichia coli* isolated from cattle feces in Bogor slaughterhouse, Indonesia. The results showed that the occurrence of 8.6% of CTX-M producers in cattle feces was identified and exhibited multidrug resistance phenotypes to at least four antibiotics [7]. ESBL/AmpC producing *E. coli* isolates that were resistant to at least three different antibiotic classes could be defined as MDR [13].

This study showed that 80% ESBL/AmpC producing *E. coli* in the environment of Bogor slaughterhouse displayed MDR to at least three different classes of antibiotics (β -lactam, aminoglycosides, fluoroquinolones, tetracycline, and folic acid inhibitor). In this work, ESBL/AmpC-producing *E. coli* isolates had high resistance against penicillin G (100.0%) and streptomycin (100.0%), followed by gentamicin (60.0%), trimethoprim-sulfamethoxazole (60.0%), tetracycline (40.0%), ciprofloxacin (40.0%), enrofloxacin (20.0%) and none of them showed resistance against polymyxin B (0.0%). Multidrug resistant ESBL/AmpC producing *E. coli* has been reported often lead the failure and limited treatment options in human and animals [8,14].

Multidrug resistance could be obtained from mobile genetic materials such as plasmids, transposons, and class I integrons. Plasmids mediating *qnr*-type resistance have ability to carry the resistant genes such as β -lactams, chloramphenicol, tetracycline, and aminoglycosides [15]. Trimethoprim-sulfamethoxazole resistance *E. coli* often correlates with the presence of dihydrofolate reductase (DHFR) and dihydropteroate synthase (DHPS) genes in integrons [16]. Another study reported that ESBL/AmpC-producing *E. coli* also showed resistant against aminoglycosides (streptomycin and gentamicin). This antibiotic has good ability against gram negative bacilli infection. The most of resistant mechanism in bacteria against aminoglycoside is enzymatic modification of antibiotic molecule [8].

The prevalence of ESBL/AmpC producing *E. coli* has also been reported increasingly. One of the reasons in this situation is improper used of quinolone antibiotics in livestock [17]. WHO and OIE were affirmed that the (fluoro) quinolones and of 3rd/4th generation cephalosporins classes are the critically important drugs between human and animals health [18]. Many studies have been conducted using molecular technique to detected resistance genes (β -lactamase and plasmid-mediated quinolone resistance (PMQR) and *E. coli* phylogenetic groups [19].

More than a half of MDR ESBL/AmpC-producing *E. coli* (60%) were found from untreated waste water in Bogor Slaughterhouse. ESBL/AmpC-producing *E. coli* from improperly treated waste could easily contaminate the ecological cycle [17]. It is necessary to improve the biosecurity and hygiene practice to reduce MDR ESBL/AmpC-producing *E. coli* contamination to the environment around slaughterhouse [12]. Responsible used of antibiotics in animals, including the 3rd/4th generation cephalosporins is highly effective to reduce the occurrence of antimicrobial resistant [20].

WHO has been declared that one step to prevent the ineffectiveness of antibiotics in humans is avoiding the use of critical important antibiotics for human in food animals production [21]. The policy of antibiotic used in livestock production and implementation of good hygiene practices in the slaughterhouse could reduce significantly the probability of MDR ESBL/AmpC producing *E. coli* contamination to the animal products and environment [10].

Most of ESBL/AmpC- producing *E. coli* (80%) isolated from the environment of Bogor slaughterhouse showed multidrug resistance phenotypes to at least three antibiotic classes. The dissemination of these organisms could be a problem of food safety, human, animals, and other pathogen bacteria.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

We thank all veterinarians and technical staff from Agency of Agricultural in Bogor, West Java, Indonesia. This study was supported by the Ministry of Research, Technology and Higher Education of the Republic of Indonesia.

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