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Public Health

Antimicrobial resistance in *Escherichia coli* isolated from brown rats and house shrews in markets, Bogor, Indonesia

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ABSTRACT. The prevalence of antimicrobial resistance (AMR) in small mammals dwelling in the city was used as an indicator of AMR bacteria in the environment. We captured 87 small mammals (79 brown rats and 8 house shrews) in four markets, Bogor, Indonesia in October 2019, and we obtained 20 AMR *Escherichia coli* (*E. coli*) from 18 brown rats and two house shrews. Of these, eight isolates were determined to be multi-drug resistant (MDR) *E. coli*, suggesting the potential contamination of AMR *E. coli* in the markets in Bogor, Indonesia, and that mammals, including humans, are at risk of infection with AMR *E. coli* from environment.

KEY WORDS: antimicrobial resistance, brown rat, *Escherichia coli*, house shrew, Indonesia

Antimicrobial resistance (AMR) is a global public health issue. Extensive use of antibiotics in humans and animals causes an increase of AMR in bacteria [2]. The Regional Resistance Surveillance Program administered by 12 Asia-Pacific countries has shown that extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* (*E. coli*) among hospitalized patients was most prevalent in Indonesia [20]. Indonesia is the country with the fourth-largest population and is infested with a rich variety of small mammals [3, 24, 29]. The prevalence of AMR in small mammals in the city was used as an indicator of AMR bacteria that potentially infect mammals present in the environment.

In Indonesia, the usage of antimicrobials is dominantly in livestock in comparison to other sectors. According to a survey conducted by the Ministry of Agriculture and Food and Agriculture Organization in 2018, most of farmers (80.0%) in broiler chicken farms in West Java, East Java and South Sulawesi routinely used antimicrobials for disease prevention and boost production [6]. The surveillance revealed only an intensive usage of antimicrobials, not the prevalence of AMR bacteria in farms. Regarding the prevalence of AMR *E. coli* infection in animals in Indonesia, a study in 2014 demonstrated that *E. coli* isolated from chickens were of the phenotype resistant to all ten examined antimicrobials. Among these, the common resistant phenotype was observed for oxytetracycline (61.5%), ampicillin (43.6%), and quinolones (35.9–42.3%) [28]. In another study, 11.8% of *E. coli* isolated from the broiler meat supply chain were colistin-resistant [19]. Moreover, ESBL-producing *E. coli* was found in 8.6% of cattle feces from slaughterhouses [26], and multi-drug resistant (MDR) *E. coli* was reported in 57.3% of swine fecal samples [14]. MDR among AMR *E. coli* was also found in 25% from Döner Kebab meat in Bogor, Indonesia [23]. These results indicated that a variety of AMR phenotypes in *E. coli* were existed and might be a widespread in the environment, Indonesia. In this study, we aimed to investigate AMR profiles and the prevalence of AMR genes in *E. coli* strains isolated from small mammals in Bogor, Indonesia.

In Indonesia, traditional markets are sold a variety of goods such as fresh and dry meat, fish, fruit and vegetables, and daily necessities. Those are sold in separate booths with roof or separate tiled booths in each market. In the urban areas, rodents and house shrews (*Suncus murinus*) are dwelling nearby food sources such as markets. They have never been purposefully treated with antimicrobials. AMR bacteria carried by these animals could reflect AMR levels in surrounding environments. In October 2019, a total of 87 small mammals were captured in four markets (Anyar, Bogor, Jambu Dua, and Merdeka) in Bogor, Indonesia, using box traps (Table 1). To collect the rectal feces, small mammals were euthanized by isoflurane inhalation, as recommended by the

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American Veterinary Medical Association (AVMA) guidelines. Seventy-nine brown rats (*Rattus norvegicus*) and eight house shrews were identified by DNA sequencing of the mitochondrial cytochrome *b* gene [18, 30]. Isolation and identification of *E. coli* from fecal samples were performed using Luria-Bertani (LB) broth (Dickinson and Co., Franklin Lakes, NJ, USA), and Deoxycholate-hydrogen sulfide-lactose (DHL) Agar (Eiken Chemical Co., Ltd., Tokyo, Japan). A colony showing typical morphology from each animal was identified to *E. coli* by the biochemical tests and detecting the *yaiO* gene by PCR, as described in our previous study [17].

Antimicrobial susceptibility tests were conducted using the Kirby-Bauer disc diffusion method, and the titer details used are as follows: ampicillin (ABP, 10 µg), cefodizime (CDZ, 30 µg), gentamicin (GM, 10 µg), tetracycline (TC, 30 µg), ciprofloxacin (CIP, 5 µg), cefotaxime (CTX, 30 µg), amoxicillin-clavulanate (ACV, 20 µg and 10 µg, respectively), nalidixic acid (NA, 30 µg), chloramphenicol (CP, 30 µg), sulfamethoxazole-trimethoprim (ST, 1.25 µg and 23.75 µg, respectively) (Eiken, Chemical Co., Ltd.).

Forty-five *E. coli* were isolated from 87 small mammals. Of which, 20 *E. coli* were identified to AMR isolates (18 isolates from brown rats and 2 isolates from house shrews). The highest prevalence of resistance was observed for TC (85%: 17/20), followed by ABP (75%: 15/20), ST (35%: 7/20), NA (30%: 6/20), CIP (20%: 4/20), CP (10%: 2/20), and GM (5%: 1/20) (Table 2). TC resistance was most frequently observed in previous studies, with rates of 78% and 50% observed in *E. coli* isolated from rodents in Hanoi, Vietnam [17] and Trinidad and Tobago [21], respectively. A previous study conducted in 1988 in Java island, Indonesia showed a 13.3% TC resistance in AMR *E. coli* isolated from rats [8]. In recent studies, the prevalence of *E. coli* with TC resistance accounted for 64–88% in hospitalized patients and 85.3% in broiler chickens in Indonesia [16, 22, 25]. In Indonesia, TC has been

Table 1. No. of antimicrobial-resistant *Escherichia coli* isolated from small mammals in Bogor, Indonesia

Location	Anyar market	Bogor market	Jambu Dua market	Merdeka market	Subtotal	Total
Latitude, Longitude	6°35'31.4"S 106°47'33.1"E	6°36'14.0"S 106°47'58.2"E	6°34'12.3"S 106°48'28.2"E	6°35'32.0"S 106°47'10.2"E		
No. of resistant isolates/ no. of rodents (%)	7/45 (15.6)	6/21 (28.6)	3/7 (42.9)	2/6 (33.3)	18/79 (22.7)	20/87 (23)
No. of resistant isolates/ no. of house shrews (%)	-	2/8 (25)	-	-	2/8 (25)	

Table 2. Prevalence of antimicrobial-resistant *Escherichia coli* isolated from small mammals in Bogor, Indonesia

Antimicrobial agents ^{a)}	Antimicrobial classes	No. of resistant isolates (%)				Subtotal	Total n=20
		Anyar market n=7	Bogor market n=8	Jambu Dua market n=3	Merdeka market n=2		
ABP	Beta-lactams	4 (57.1)	7 (87.5) ^{b)}	3 (100)	1 (50)	15 (75)	15 (75)
ACV		0	0	0	0	0	
CDZ		0	0	0	0	0	
CTX		0	0	0	0	0	
CIP	Quinolone	1 (14.3)	2 (25)	1 (33.3)	0	4 (20)	6 (30)
NA		2 (28.6)	2 (25)	1 (33.3)	1 (50)	6 (30)	
CP	Chloramphenicol	0	1 (12.5) ^{b)}	1 (33.3)	0	-	2 (10)
GM	Aminoglycoside	0	0	1 (33.3)	0	-	1 (5)
ST	Sulfonamide	0	5 (62.5) ^{b)}	2 (66.7)	0	-	7 (35)
TC	Tetracycline	6 (85.7)	8 (100) ^{b)}	2 (66.7)	1 (50)	-	17 (85)
Multi-drug resistant		1 (14.3)	5 (62.5) ^{b)}	2 (66.7)	0	-	8 (40) ^{b)}

a) ABP: ampicillin, ACV: amoxicillin-clavulanate, CDZ: cefodizime, CTX: cefotaxime, CIP: ciprofloxacin, NA: nalidixic acid, CP: chloramphenicol, GM: gentamicin, ST: sulfamethoxazole-trimethoprim, TC: tetracycline. b) Including one isolate from a house shrew.

Table 3. Characteristic of multi-drug resistant *Escherichia coli* isolated from rodents and house shrews in Bogor, Indonesia

ID	Location	Animal species	Antimicrobial resistant phenotypes ^{a)}	Antimicrobial resistance genes
BG-44	Anyar market	<i>Rattus norvegicus</i>	ABP-NA-TC	<i>bla</i> _{TEM} - <i>tet</i> (A)
BG-69	Jambu market	<i>Rattus norvegicus</i>	ABP-CIP-NA-CP-TC-ST	<i>bla</i> _{TEM} - <i>tet</i> (A)- <i>sul3</i>
BG-74	Jambu market	<i>Rattus norvegicus</i>	ABP-GM-ST	<i>bla</i> _{TEM} - <i>sul3</i>
BG-77	Bogor market	<i>Rattus norvegicus</i>	ABP-TC-ST	<i>bla</i> _{TEM} - <i>tet</i> (B)- <i>sul3</i>
BG-78	Bogor market	<i>Rattus norvegicus</i>	ABP-CIP-NA-TC-ST	<i>bla</i> _{TEM} - <i>tet</i> (A)- <i>sul3</i>
BG-79	Bogor market	<i>Rattus norvegicus</i>	ABP-CIP-NA-TC-ST	<i>bla</i> _{TEM} - <i>tet</i> (A)- <i>sul3</i>
BG-80	Bogor market	<i>Rattus norvegicus</i>	ABP-TC-ST	<i>bla</i> _{TEM} - <i>tet</i> (B)- <i>sul3</i>
BG-83	Bogor market	<i>Suncus murinus</i>	ABP-CP-TC-ST	<i>bla</i> _{TEM} - <i>tet</i> (B)- <i>sul3</i>

a) ABP: ampicillin, CIP: ciprofloxacin, NA: nalidixic acid, CP: chloramphenicol, GM: gentamicin, ST: sulfamethoxazole-trimethoprim, TC: tetracycline.

Table 4. No. of antimicrobial resistance genes detected in 20 antimicrobial-resistant *Escherichia coli* isolated from small mammals in Bogor, Indonesia

AMR genes	Antimicrobial classes	No. of isolates positive for antimicrobial resistance (AMR) genes (%)					
		Anyar market n=7	Bogor market n=8	Jambu Dua market n=3	Merdeka market n=2	Subtotal	Total n=20
<i>bla</i> _{TEM}	Beta-lactams	4 (57.1)	7 (87.5)	3 (100)	1 (50)	15 (75)	15 (75)
<i>bla</i> _{CTX-M}		0	0	0	0	0	
<i>bla</i> _{CMY-2}		0	0	0	0	0	
<i>bla</i> _{SHV}		0	0	0	0	0	
<i>qnrA</i>	Quinolone	0	0	0	0	-	0
<i>sul1</i>	Sulfonamide	0	0	0	0	0	8 (40)
<i>sul2</i>		0	0	0	0	0	
<i>sul3</i>		1 (14.3)	5 (62.5)	2 (66.7)	0	8 (40)	
<i>tet(A)</i>	Tetracycline	6 (85.7)	5 (62.5)	2 (66.7)	1 (50)	14 (70)	17 (85)
<i>tet(B)</i>		0	3 (37.5)	0	0	3 (15)	
<i>tet(C)</i>		0	0	0	0	0	

widely used in the livestock industry and is also often used for self-medication in humans, which may have led to the increase in the contamination of TC resistant *E. coli* in the environment [8, 11, 16]. In this study, the prevalence of ABP resistant *E. coli* was 75%, which was greater than that reported in a study in 1988, showing 20% of ABP resistant *E. coli* isolated from rats [8]. Other reports showed that the observed prevalence of ampicillin resistant *E. coli* was 90% in rodents from England and 25% in mice, vole and shrews from Canada [7, 15]. The prevalence of ABP resistance in this study is similar to that observed in hospital settings in Indonesia, which ranged from 73 to 78% [5, 25]. Therefore, our results suggest the possibility that small mammals acquire AMR *E. coli* through contact with human sewage and animal excretions containing AMR *E. coli* in the environment.

Eight *E. coli* isolates (40%) were identified as MDR, having resistance to at least three antimicrobial classes (Table 3). Of these, seven MDR *E. coli* were isolated from brown rats and one isolate was isolated from a house shrew (Table 3). In 1988, of *E. coli* isolated from rats in Indonesia, 20% of the AMR *E. coli* were identified as MDR. The prevalence of MDR *E. coli* in this study is similar to those reported for rodents in Vancouver, Canada (41.5%) [12] and Berlin, Germany (58.2%) [9]. In the recent another study in Indonesia, the prevalence of MDR *E. coli* isolated from swine (57.3%) was also similar to our study [14]. The prevalence of MDR *E. coli* in hospitalized patients (71%) was quite high compared to our results [20]. These results indicate that the prevalence of MDR *E. coli* had increased in the environment in Indonesia.

Next, we examined the resistance genes in the AMR *E. coli* isolates. The major β -lactamase genes (*bla*_{CTX-M} group (*bla*_{CTX-M-1}, *bla*_{CTX-M-2}, *bla*_{CTX-M-8/25}, *bla*_{CTX-M-9}), *bla*_{TEM}, *bla*_{SHV} and *bla*_{CMY-2}), sulfonamides (*sul1*, *sul2* and *sul3*), quinolone (*qnrA*) and tetracycline [*tet(A)*, *tet(B)* and *tet(C)*] were tested by single or multiplex PCR [17]. The most frequently detected resistance gene was *bla*_{TEM} (75%: 15/20), followed by *tet(A)* (70%: 14/20), *sul3* (40%: 8/20) and *tet(B)* (15%: 3/20) (Table 4). All *E. coli* resistant to ABP and TC corresponded to the *E. coli* carrying *bla*_{TEM} and tet (A) and (B) genes, respectively. Other resistance genes were not detected in any isolates, with the exception of *sul3* (Table 4). Seven out of the eight AMR *E. coli* carrying the *sul3* gene were resistant to ST, but the remaining one was susceptible to this antibiotic. In the isolates, ST susceptibility might be attributed to trimethoprim susceptibility rather than to sulfamethoxazole [10]. Six AMR *E. coli* with the phenotype resistant to quinolones, did not harbor the *qnrA* gene, suggesting that quinolone resistance in *E. coli* isolated from small mammals in Indonesia may be caused by other mechanisms such as chromosomal mutations or efflux pumps [13].

Indonesia has been reported as the country with the highest prevalence of ESBL-producing *E. coli* and *Klebsiella* spp. in clinical cases [20]. A previous study also reported ESBL-producing *E. coli* (8.6%) isolated from cattle in Bogor, Indonesia [26]. Another study showed a prevalence of 14.3% ESBL-producing *E. coli* isolated from environmental samples in slaughterhouses, Bogor [27]. In this study, there were no ESBL-producing *E. coli* found in small mammals. Moreover, the mobilized colistin resistance genes (*mcr-1* to *-3*) were not detected in the AMR *E. coli* isolates. Since the ESBL enzyme family and *mcr* genes are located on plasmids [1, 4], our data suggested that these plasmids may not be widely distributed in the environment of Bogor, Indonesia.

In summary, 20 AMR *E. coli*, including eight MDR *E. coli*, were isolated from the feces of small mammals captured in Bogor, Indonesia. Small mammals dwelling in the city are more likely to be exposed to human sewage, probably resulting in the acquisition of AMR *E. coli* from their surrounding environment. Although the public health risk posed by small mammals carrying AMR *E. coli* remains unclear, our data shows that compared to data from 1988, AMR profiles of *E. coli* isolated from small mammals and MDR *E. coli* contamination in the environment of Bogor, Indonesia had increased.

POTENTIAL CONFLICTS OF INTEREST. The authors have nothing to disclose.

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