The Journal of Veterinary Medical Science

FULL PAPER

Public Health

Antibiotic-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam

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ABSTRACT. Antimicrobial resistance (AMR) is a global public health concern for both clinical and veterinary medicine. Rodent feces are one of the major infectious sources of zoonotic pathogens including AMR bacteria. So far, there are limited studies reported focused on *Escherichia coli* isolated in rodent feces from rural and suburban areas in Vietnam. In this study, we investigated the prevalence of antimicrobial resistance in *E. coli* isolated from feces samples of 144 urban rodents caught in Hanoi, Vietnam. A total of 59 AMR *E. coli* was isolated from urban rodents of which 42 were multidrug-resistant (MDR) isolates (resistance to at least three classes of antimicrobial agents), four were extended-spectrum β-lactamase (ESBL) producing isolates and five were colistin-resistant isolates. The highest prevalence of the resistance was against ampicillin (79.7%: 47/59), followed by tetracycline (78.0%: 46/59), nalidixic acid (67.8%: 40/59), sulfamethoxazole-trimethoprim (59.3%: 35/59), chloramphenicol (45.8%: 27/59), ciprofloxacin (44.1%: 26/59), cefotaxime (30.5%: 18/59), cefodizime (23.7%: 14/59), amoxicillin-clavulanate (22.0%: 13/59), and gentamicin (22.0%: 13/59). With regard to the virulence genes associated with diarrheagenic *E. coli* (DEC), only *aaiC* gene found in one AMR isolate. In general, the use of antimicrobials does not aim to treat rodents except for companion animals. However, our findings show the carriage of AMR and MDR *E. coli* in urban rodents and highlight the potential risk of rodents in Hanoi acting as a reservoir of transferable MDR *E. coli*, including ESBL-producing, colistin-resistant *E.* coli, and virulence-associated with DEC.

KEY WORDS: antimicrobial-resistant *Escherichia coli*, multidrug-resistant *Escherichia coli*, rodent, Vietnam

J. Vet. Med. Sci.
82(5): 653–660, 2020
doi: 10.1292/jvms.19-0697

Received: 5 January 2020
Accepted: 19 March 2020
Advanced Epub:
30 March 2020

Rodents are known as reservoir animals for the transmission of a variety of zoonotic pathogens in urban locations [14, 22]. Some bacteria, viruses and parasites have been carried by rodents, namely *Escherichia coli*, *Salmonella* spp., *Campylobacter* spp., *Leptospira* spp., *Orientia tsutsugamushi* and hantavirus [18, 26, 28, 41]. Rodent-borne diseases spread to humans through the consumption of contaminated food, the inhalation of the aerosol, rodent bites and arthropod vectors from rodents [33]. Feces are one of the significant sources for pathogens including *E. coli*, with the potential risk for causing intestinal diseases in humans and animals [20]. In comparison to livestock, a few studies have shown a profile of AMR *E. coli* carried by rodents in both urban and rural areas [7, 18, 23, 40]. Most of the previous studies focused only on the resistant phenotype of AMR *E. coli* isolated from rodents [7, 17, 41]. Recently, the research has shifted to AMR genes in combination with the resistant phenotype [18]. Although a discrepancy sometimes occurs in the results between the phenotype and genotype, it is able to conduct broader analysis predictions.

In the zoonotic perspective, AMR *E. coli* and diarrheagenic *E. coli* are of significant concerns. The recent reports studied on the potential zoonotic *E. coli* were indicated the high prevalence of AMR *E. coli* including ESBL-producing *E. coli* and virulence genes carried *E. coli* from urban rodents [6, 18, 19, 24]. In addition, colistin-resistant bacteria have gained much more attention

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since the first mobile colistin resistance gene (*mcr-1*) was identified in *E. coli* in China 2015 [31]. Colistin-resistant bacteria have been reported from humans, livestock and environments in various locations in the world [9, 50]. AMR genes including the colistin resistance genes carried by *E. coli* can be transferred to other bacterial populations, rising a potential risk of the expansion to humans and the environment. Rodents inhabiting close to humans and livestock may involve in the transmission of AMR genes including the colistin resistance genes. There was a study which investigated the prevalence of AMR *E. coli* including ESBL-producing *E. coli* isolated from small mammals in the Mekong Delta, Vietnam [40]. The prevalence of MDR was approximately eight times higher in *E. coli* isolated from small mammals (rodents and shrews) trapped on farms than those trapped in forests or rice fields. The results strongly suggested that MDR in small mammals were obtained from MDR-carried livestock and their environment.

Since rodents have the ability of the adaption to their habitat particularly with regards to a density of human population, rodents are in contact with human waste and infrastructure (e.g., garbage and sewage) in the urban areas. In this study, we investigated the prevalence of putative pathogenic *E. coli* in rodents trapped in Hanoi, Vietnam and then, examined the possible association and distribution of antimicrobial resistant profiles, antibiotic resistance genes, ESBL-producing, virulence genes and colistin-resistance in *E. coli* isolates from urban rodents.

MATERIALS AND METHODS

Rodent trapping and sample collection

Rodents were captured using live traps at eight locations of Hanoi, Vietnam in October 2017, March and June 2018 (Table 1). The identification of rodent species was conducted by DNA sequencing of the mitochondrial cytochrome *b* gene [60]. Rodents were euthanized by isoflurane inhalation, as recommended by the American Veterinary Medical Association (AVMA) guidelines. Rectal swabs and/or rectal feces were collected and frozen using dry ice and then stored at −80°C until use [11].

E. coli isolation

Rectal swabs and/or feces were soaked in 1 m*l* of Luria-Bertani (LB) broth (Dickinson and Co., Franklin Lakes, NJ, USA). The samples were plated using full loops (10 mm inoculate loop) onto Deoxycholate Hydrogen Sulfide Lactose (DHL) Agar (Eiken Chemical Co., Ltd., Tokyo, Japan), and incubated at 37°C overnight. One colony showing typical *E. coli* morphology from each rat sample was identified by the following biochemical tests: Triple Sugar Iron (TSI) agar (Becton, Dickinson and Co.), Lysine Indole Motility (LIM) test (Eiken Chemical Co., Ltd.), Voges-Proskauer (VP) test (Eiken Chemical Co., Ltd.), citrate utilization tests, and oxidase test (Merck KGaA, Darmstadt, Germany) [55]. The confirmation of *E. coli* was conducted by detecting the *yaiO* gene by PCR [35].

Antimicrobial susceptibility test

Antimicrobial susceptibility tests were conducted on Mueller Hinton II agar (Becton, Dickinson and Co.) plates according to the Kirby-Bauer disc diffusion method using disc and titer details 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). Potential production of extended-spectrum β-lactamase (ESBL) was confirmed by the double-disk synergy test using CTX, ACV and Ceftazidime (CAZ, 30 μg). The resistance phenotype was interpreted accurately according to the Clinical and Laboratory Standards Institute (CLSI) guidelines 2011 and the manufacturer guideline [12]. In this study, *E. coli* JCM 384 was used as a quality control strain.

Detection of antimicrobial resistance genes

Colonies of isolated *E. coli* were dissolved in 100 μ*l* of distilled water and boiled at 95°C for 15 min. The major resistant genes for β-lactamase (bla_{TEM}, bla_{SHV}, bla_{CTX-M} and bla_{CMY-2}), sulfonamides (*sul1*, *sul2* and *sul3*), quinolone (*qnrA*) and tetracycline

Table 1. No. of antimicrobial-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam

Location		GTVT hospital			Ha Dong hospital		Dong Tam market	Thanh Cong market		Ha Dong market		Phung Khoang market	Thai Ha market		Giap Bat cargo station
Latitude, Longitude		21°1'33.50"N, 105°48'11.17"E			20°58'17.16"N, 105°46'30.66"E		20°59'48.52"N, 105°50'42.56"E	21°1'21.51"N, 105°48'54.17"E		20°58'11.61"N, 105°46'46.86"E		20°59'11.32"N, 105°47'37.87"E	21°0'49.40"N, 105°49'20.76"E		20°58'48.77"N, 105°50'29.24"E
Rat species		Rn[a)]	Ra[b)]	Rr[c)]	Rn[a)]	Rr[c)]	Rn[a)]	Rn[a)]	Rr[c)]	Rn[a)]	Rr[c)]	Rn[a)]	Rn[a)]	Rr[c)]	Rn[a)]
No. of resistant isolates / no. of samples (%)	Oct. 2017	0/9	0/1	0/1	-	-	3/12	1/17	-	-	-	-	-	-	3/16
	Mar. 2018	-	-	-	12/13	0/2	-	6/15	-	3/12	1/2	-	-	-	6/11
	Jun. 2018	2/5	-	-	-	-	-	8/9	0/1	2/2	-	10/10	2/4	0/2	-
	Subtotal	2/14	0/1	0/1	12/13	0/2	3/12	15/41	0/1	5/14	1/2	10/10	2/4	0/2	9/27
	Location total	14/31 (45.2)					36/86 (41.9)								9/27 (33.3)
	Total	59/144 (41)													

a) *Rattus norvegicus*, b) *Rattus argentiventer*, c) *Rattus rattus.*

[*tet*(A), *tet*(B) and *tet*(C)] were tested by single or multiplex PCR. To classify bla_{CTX-M} genes into the five major groups, CTX-M-1, CTX-M-2 (TOHO), CTX-M-8/25 and CTX-M-9 groups, the detection of bla_{CTX-M} genes were used four primer sets designed by Pitout *et al*. to detect [43]. PCR conditions and primers (Life Technologies Japan Ltd.) for other resistance genes described by Kozak *et al*. and Mammeri *et al*. [29, 32] were used in this study.

Identification of diarrheagenic E. coli

Primers of multiplex PCR used in this study were described in Table 2. The target genes associated with diarrheagenic *E. coli* (DEC) include *elt* (heat labile enterotoxin), *est* (heat stable enterotoxin), *bfpA* (bundle-forming pilus), *eae* (*E. coli* attaching and effacing), *stx* (Shiga toxin), *ipaH* (invasion plasmid antigen H), *aatA* (outer membrane protein), *aaiC* (secreted protein) and *aggR* (transcriptional activator).

The PCR mixture was adjusted to 25 μl containing with of 12.5 μl of Gotaq (Promega Corp., Madison, WI, USA), 0.3 μM of each primer, distilled water and 2 μl of DNA template. Multiplex PCR was performed as follows; 95°C for 5 min, 30 cycles of 95°C for 20 sec, 52°C for 40 sec, 72°C for 30 sec and 72°C for 7 min.

Detection of colistin-resistant E. coli

Detection of colistin resistance genes was performed by PCR using the primers for *mcr-1*, *mcr-2* and *mcr-3* genes as described in the previous studies [31, 58, 59]. Amplified fragments were confirmed by DNA sequencing. The sequences were deposited in the GenBank (accession numbers MN519790–MN519794). Susceptibility to colistin was examined by the broth microdilution and macro dilution methods. The determination of minimal inhibitory concentration (MIC) was >2 mg/*l* as resistant according to the breakpoint for *Enterobacteriaceae* in the European Committee on Antimicrobial Susceptibility Testing (EUCAST).

RESULTS

Prevalence of resistant E. coli isolates from urban rodents

A total of 144 rodents (ricefield rat (*Rattus argentiventer*); 1, brown rat (*R. norvegicus*); 135, black rat (*R. rattus*); 8) were captured at eight locations in urban areas of Hanoi in 2017 and 2018 (Table 1). Fifty-nine isolates (58 isolates from *R. norvegicus* and one isolate from *R. rattus*) were identified to AMR *E. coli*. AMR *E. coli* were obtained from 14 out of 31 (45.2%) from hospitals, 36 of 86 (41.9%) from markets and 9 of 27 (33.3%) from a cargo station. There was no statistical difference in the prevalence of AMR *E. coli* between hospitals, markets and a cargo station. The most common AMR was resistance to ABP, which was detected in 79.7% (47/59), followed by TC (78.0%: 46/59), NA (67.8%: 40/59), ST (59.3%: 35/59), CP (45.8%: 27/59), CIP (44.1%: 26/59) and CTX (30.5%: 18/59) (Table 3). Multidrug resistance (MDR; resistance to three or more antimicrobial classes) were identified in 42 isolates out of 59 (71.2%) from *R. norvegicus*.

Table 2. List of primers for the identification of diarrheagenic *Escherichia coli*

Pathogen[a)]	Primer name	Target gene	Primer sequence (5′-3′)	Amplicon (bp)	Reference
ETEC	LT-F LT-R	*elt*	CACACGGAGCTCCTCAGTC CCCCCAGCCTAGCTTAGTTT	508	Panchalingam *et al*. 2012
	ST-F ST-R	*est*	GCTAAACCAGTAG/AGGTCTTCAAAA CCCGGTACAG/AGCAGGATTACAACA	147	Panchalingam *et al*. 2012
EPEC	BFPA-F BFPA-R	*bfpA*	GGAAGTCAAATTCATGGGGG GGAATCAGACGCAGACTGGT	367	Panchalingam *et al*. 2012
	SK1 SK2	*eae*	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	881	Toma *et al*. 2003
STEC	VTcom-u VTcom-d	*stx*	GAGCGAAATAATTTATATGTG TGATGATGGCAATTCAGTAT	518	Toma *et al*. 2003
	SK1 SK2	*eae*	CCCGAATTCGGCACAAGCATAAGC CCCGGATCCGTCTCGCCAGTATTCG	881	Toma *et al*. 2003
EIEC	ipaIII ipaIV	*ipaH*	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	619	Toma *et al*. 2003
	CVD432F CVD432R	*aatA*	CTGGCGAAAGACTGTATCAT CAATGTATAGAAATCCGCTGTT	630	Panchalingam *et al*. 2012
EAEC	AAIC F AAIC R	*aaiC*	ATTGTCCTCAGGCATTTCAC ACGACACCCCTGATAAACAA	215	Panchalingam *et al*. 2012
	aggRks1 aggRks2	*aggR*	GTATACACAAAAGAAGGAAGC ACAGAATCGTCAGCATCAGC	254	Toma *et al*. 2003

a) ETEC: Enterotoxigenic *E. coli*, EPEC: Enteropathogenic *E. coli*, STEC: Shiga toxin-producing *E. coli*, EIEC: Enteroinvasive *E. coli*; EAEC: Enteroaggregative *E. coli.*

Detection of AMR genes

The most frequent resistance gene was bla_{TEM} (69.5%: 41/59) followed by *tet*(A) (64.4%: 38/59), *sul2* (35.6%: 21/59), *sul3* (23.7%: 14/59), *sul1* (18.6%: 11/59), and *tet*(B) (11.9%: 7/59) (Table 4). On the other hand, genes of bla_{SHV}, *qnrA* and *tet*(C) were not detected in any isolates. Nine of the 50 isolates phenotypically resistant to *β*-lactams (ampicillin, amoxicillin-clavulanate, cefodizime, and/or cefotaxime) had none of *β*-lactamase genes (Tables 3 and 4). In isolates showing quinolone-resistant phenotypes (26 and 40 isolates resistant to ciprofloxacin and nalidixic acid, respectively), no quinolone-resistance gene (*qnrA*) was found. Four out of the 35 isolates phenotypically resistant to sulfamethoxazole-trimethoprim had no genes of sulfonamide resistance, and vice versa. Two out of the 46 isolates phenotypically resistant to tetracycline possessed no tetracycline resistance genes.

Detection of ESBL-producing E. coli

According to the double-disk synergy test using CTX, ACV and CAZ, four out of the 59 isolates were confirmed as ESBL-producing *E. coli* (Table 5). Three out of the four ESBL-producing *E. coli* were isolated from Ha Dong hospital and one from Ha Dong market. Furthermore, all four ESBL-producing isolates were identified as MDR and carried the bla_{TEM} gene, and three out of these isolates harbored $bla_{CTX\text{-}M\text{-}1}$ group.

Detection of diarrheagenic genes

Isolates were analyzed for the presence of virulence genes associated with enterotoxigenic *E. coli* (ETEC), enteropathogenic

Table 3. Prevalence of antimicrobial-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam

Antimicrobial agents[a)]	Antimicrobial classes	No. of resistant isolates (%)									
		GTVT hospital n=2	Ha Dong hospital n=12	Dong Tam market n=3	Thanh Cong market n=15	Ha Dong market n=6	Phung Khoang market n=10	Thai Ha market n=2	Giap Bat cargo station n=9	Subtotal	Total n=59
ABP	Beta-lactams	2 (100)	11 (91.7)	2 (66.7)	12 (80)	5[b)] (83.3)	8 (80)	1 (50)	6 (66.7)	47 (79.7)	50 (84.7)
ACV		0	2 (16.7)	0	4 (26.7)	2 (33.3)	4 (40)	0	1 (11.1)	13 (22.0)	
CDZ		0	3 (25)	0	2 (13.3)	1 (16.7)	7 (70)	0	1 (11.1)	14 (23.7)	
CTX		0	4 (33.3)	1 (33.3)	1 (6.7)	2 (33.3)	7 (70)	0	3 (33.3)	18 (30.5)	
CIP	Quinolone	1 (50)	7 (58.3)	0	7 (46.7)	3 (50)	7 (70)	0	1 (11.1)	26 (44.1)	43 (72.9)
NA		0	10 (83.3)	1 (33.3)	11 (73.3)	4 (66.7)	9 (90)	1 (50)	4 (44.4)	40 (67.8)	
CP	Chloramphenicol	1 (50)	7 (58.3)	1 (33.3)	6 (0.4)	3 (50)	7 (70)	0	2 (22.2)	-	27 (45.8)
GM	Aminoglycoside	0	5 (41.7)	0	5 (33.3)	1 (16.7)	2 (20)	0	0 (0)	-	13 (22.0)
ST	Sulfonamide	2 (100)	9 (75)	1 (33.3)	9 (60)	3 (50)	9 (90)	0	2 (22.2)	-	35 (59.3)
TC	Tetracycline	2 (100)	9 (75)	2 (66.7)	14 (93.3)	5[b)] (83.3)	9 (90)	0	5 (55.6)	-	46 (78.0)
Multi-drug resistant		2 (100)	10 (83.3)	1 (33.3)	12 (80)	4 (66.7)	9 (90)	0	4 (44.4)	-	42 (71.2)

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 an isolate from *Rattus rattus.*

Table 4. No. of antimicrobial resistance genes detected in 59 antimicrobial-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam

AMR genes	Antimicrobial classes	No. of isolates positive for AMR genes (%)									
		GTVT hospital n=2	Ha Dong hospital n=12	Dong Tam market n=3	Thanh Cong market n=15	Ha Dong market n=6	Phung Khoang market n=10	Thai Ha market n=2	Giap Bat cargo station n=9	Subtotal	Total n=59
bla_{TEM}	Beta-lactams	2 (100)	10 (83.3)	1 (33.3)	12 (80)	4[a)] (66.7)	8 (80)	0	4 (44.4)	41 (69.5)	41 (69.5)
$bla_{CTX\text{-}M\text{-}1}$ group		0	2 (16.7)	0	0	1 (16.7)	0	0	0	3 (5.1)	
$bla_{CMY\text{-}2}$		0	0	0	1 (6.7)	0	0	0	0	1 (1.7)	
bla_{SHV}		0	0	0	0	0	0	0	0	0 (0)	
qnrA	Quinolone	0	0	0	0	0	0	0	0	-	0 (0)
sul1	Sulfonamide	0	3 (25)	0	7 (46.7)	0	1 (10)	0	0	11 (18.6)	35 (59.3)
sul2		2 (100)	5 (41.7)	1 (33.3)	7 (46.7)	3 (50)	2 (20)	0	1 (11.1)	21 (35.6)	
sul3		1 (50)	5 (41.7)	1 (33.3)	2 (13.3)	1 (16.7)	2 (20)	0	2 (22.2)	14 (23.7)	
tet (A)	Tetracycline	2 (100)	8 (66.7)	2 (66.7)	9 (60)	4[a)] (66.7)	9 (90)	0	4 (44.4)	38 (64.4)	44 (74.6)
tet (B)		0	1 (8.3)	0	5 (33.3)	1 (16.7)	0	0	0	7 (11.9)	
tet (C)		0	0	0	0	0	0	0	0	0	

a) Including an isolate from *Rattus rattus.*

E. coli (EPEC), Shiga toxin-producing *E. coli* (STEC), enteroinvasive *E. coli* (EIEC) and enteroaggregative *E. coli* (EAEC) by multiplex PCR (Table 2). There was no detection of ETEC, EPEC, STEC and EIEC in this study. Only one out of the 59 AMR isolates carried *aaiC* gene (data not shown), indicating the potential pathogen of EAEC. The isolate carried *aaiC* gene was MDR (ABP-NA-ST-TC) and carried AMR genes of bla_{TEM}, *sul2* and *tet*(A).

Detection of colistin-resistant E. coli

The *mcr-1* genes were amplified from five isolates, 250 bp from IDs 71 and 109 and 259 bp from IDs 102, 120 and 137 (accession nos. MN519790– MN519794). These sequences were identical with the *mcr-1* gene from *E. coli* strain SHP45 (accession no. KP347127) isolated from swine in China, 2015, which was the first report of colistin-resistant *E. coli*. All of the isolates carrying *mcr-1* gene exhibited MIC of 4 μg/m*l* and showed 5 to 6 antimicrobial classes in the resistant phenotypes. The isolate ID 71 from Thanh Cong market showed resistance to all 10 antimicrobial agents tested in this study.

DISCUSSION

The present study demonstrated that 59 out of 144 (41%) rodents carried AMR *E. coli* in the urban city of Hanoi, Vietnam (Tables 1 and 2). The prevalence of AMR *E. coli* was no significant difference between hospitals, markets and the cargo station in Hanoi (Table 2). Among 59 AMR *E. coli*, the most common resistant phenotype was observed to β-lactams (84.7%), followed by tetracycline (78%), quinolone (72.9%), and sulfonamide (59.3%). The resistant to ampicillin was the most prevalent in Hanoi (79.7%) which was similar to the previous study in the Mekong Delta (85.9%) [40]. The resistance to tetracycline was much higher in Hanoi (78%) than in Mekong delta (34.5%), indicating environmental contamination with tetracycline in the urban area [54]. MDR was identified in 42 out of 59 AMR isolates (71.2%). The prevalence of MDR isolates from small mammals in Hanoi was higher than those observed in Vancouver, Canada (41.5%), Berlin, Germany (58.2%), Nairobi, Kenya (66.7%), or Mekong Delta, Vietnam (27.2%) [17, 18, 23, 40]. Since rodents in urban areas have opportunities to contact with human sewage systems and garbage dumps, they might take up with MDR *E. coli* including colistin-resistant via these routes. In recent years, colistin-resistant bacteria have been observed in wildlife and investigated to understand the mechanism of spreading colistin resistance globally. Although colistin-resistant *E. coli* were found in some wildlife, Algerian hedgehog and Barbary macaques, birds and rodents were concerned as potential carriers and/ or spreaders of colistin-resistant *E. coli* [1, 2, 4, 13, 34, 45, 47]. Our data revealed that urban rodents could be a carrier and spreader of colistin-resistant *E. coli* (Table 6). The prevalence of colistin-resistant *E. coli* in urban rodents (5/59, 8.5%) was much lower than those in healthy persons (80.6%) in Vietnam [59]. Besides colistin-resistant *E. coli*, ESBL-producing *E. coli* were isolated in urban rodents (Table 5). Prevalence of ESBL-producing *E. coli* in urban rodents (4/59, 6.8%) was also lower than livestock (20%) and healthy persons (35.2%) in Vietnam [39]. Since the role of wildlife has been documented in spreading AMR bacteria, the urban rodents may play as the maintenance host or vector for ESBL-producing and colistin-resistant isolates [53, 57].

Regarding bla_{CTX-M} genes, there are several reports of the geographically major groups. The $bla_{CTX-M-1}$ group is more common in *E. coli* isolated from human and livestock samples in Europe [8, 15]. The samples from rodent feces were also detected the $bla_{CTX-M-1}$ group in Germany [15]. In Southeast Asian countries such as Thailand, Laos and Vietnam, the $bla_{CTX-M-1}$ and bla_{CTXM-9} groups are the most predominant groups carried by *E. coli* isolated from humans [37]. On the other hand, the $bla_{CTX-M-2}$ group is

Table 5. Characteristics of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam

ID	Location	Antimicrobial resistant phenotype[a)]	β-lactamase gene
100	Ha Dong hospital	ABP-CDZ-CTX-CIP-NA-CP-GM-TC-ST	bla_{TEM}, $bla_{CTX-M-1}$ group
101		ABP-CDZ-CTX-CIP-NA-CP-ST	bla_{TEM}, $bla_{CTX-M-1}$ group
105		ABP-CDZ-CTX-CIP-NA-TC	bla_{TEM}
133	Ha Dong market	ABP-CDZ-CTX-CIP-NA-CP-GM-TC-ST	bla_{TEM}, $bla_{CTX-M-1}$ group

a) ABP: Ampicillin, ACV: Amoxicillin−Clavulanate, CDZ: Cefodizime, CTX: Cefotaxime, CIP: Ciprofloxacin, NA: Nalidixic acid, CP: Chloramphenicol, GM: Gentamicin, ST: Sulfamethoxazole, TC: Tetracycline.

Table 6. Characteristics of colistin-resistant *Escherichia coli* isolated from urban rodents in Hanoi, Vietnam

ID	Location	Colistin resistance gene	MIC[a)] (μg/m*l*)	Other resistant phenotype[b)]
71	Thanh Cong market	*mcr-1*	4	ABP-ACV-CDZ-CTX-CIP-NA-CP-GM-ST-TC
102	Ha Dong hospital	*mcr-1*	4	ABP-CIP-NA-CP-GM-ST-TC
109	Ha Dong hospital	*mcr-1*	4	ABP-CTX-NA-CP-GM-ST-TC
120	Thanh Cong market	*mcr-1*	4	ABP-CIP-NA-CP-ST-TC
137	Phung Khoang market	*mcr-1*	4	ABP-CIP-NA-CP-ST-TC

a) Minimal Inhibitory Concentration (MIC). b) ABP: Ampicillin, ACV: Amoxicillin−Clavulanate, CDZ: Cefodizime, CTX: Cefotaxime, CIP: Ciprofloxacin, NA: Nalidixic acid, CP: Chloramphenicol, GM: Gentamicin, ST: Sulfamethoxazole, TC: Tetracycline.

present in *E. coli* isolated from humans and broiler chickens in South American countries [16, 44, 49], cattle in Japan [48] and humans in Israel [10]. The $bla_{\text{CTX-M-8}}$ group was found in *E. coli* isolated from chicken meats in Brazil [36], humans in the United States [30] and gulls in Spain [52]. The $bla_{\text{CTX-M-25}}$ group was reported from clinical samples in Israel [56] and broiler chickens in Japan [61]. In this study, we found the $bla_{\text{CTX-M-1}}$ group in three ESBL-producing *E. coli* isolated from rodent feces. This is the first report of the $bla_{\text{CTX-M-1}}$ group among rodents in Asian countries.

There were reports of the discrepancies between AMR phenotypes and the presence or absence of resistance genes [3, 29]. In this study, there were almost good agreements between AMR phenotypes and the presence or absence of resistance genes in regard to the resistance to β-lactams, sulfamethoxazole-trimethoprim and tetracycline resistance. Isolates resistant to β-lactams but without β-lactamase genes (bla_{TEM}, bla_{SHV}, $bla_{\text{CTX-M}}$, $bla_{\text{CMY-2}}$) may be caused by other genes because β-lactamase genes were assigned to nine distinct structures including TEM, SHV, CTX-M, PER, VEB, GES, BES, TLA and OXA based on the comparison of their amino acid sequences [5]. AMR genes are encoded in plasmids which are responsible for the spread of resistance genes [27, 51]. Possible of horizontal transmission of β-lactamase gene ($bla_{\text{CTX-M-1}}$ group, $bla_{\text{CMY-2}}$) and colistin resistance gene (*mcr-1*) poses a risk to public health, food safety, and water source. However, there was no quinolone resistance gene (*qnrA*) in quinolone-resistant phenotypes, 26 and 40 isolates resistant to ciprofloxacin and nalidixic acid, respectively. These results may reflect the fact that resistance to these antimicrobials can be acquired by different AMR genes or another resistance mechanism such as mutations in the quinolone resistance-determining regions [18] or in efflux pumps [25].

With regard to virulence genes associated with DEC, the *aaiC* gene was detected from one MDR isolate. The *aaiC* gene is a virulence gene typically associated with enteroaggregative *Escherichia coli* (EAEC) [42]. The EAEC group have been found in diarrhea patients in several regions of the world [21]. While one of 59 isolates (1.7%) carried a virulence gene in this study, the prevalence of virulence genes in urban rodents were 0% and 3.8% in Berlin, Germany and Vancouver, Canada, respectively [18, 23]. In contrast, isolates from livestock showed higher carriage of virulence genes, 31.3% and 10.9% from calves and chicken in Vietnam, respectively [38, 46]. The low prevalence suggests a low risk of pathogenic *E. coli* infection from urban rodents, but continuous monitoring should be implemented for the risk assessment.

In conclusion, our data suggest that urban rodents could be a reservoir and spreader of AMR *E. coli* including multidrug-resistant, ESBL-producing and colistin-resistant isolates in Hanoi, Vietnam. The prevalence of AMR *E. coli* in rodents from urban areas (41%) was almost same with that in rodents from farms (45%) and much higher than forest and rice fields (5.8%) in Vietnam [40]. The risk of urban rodents carrying AMR *E. coli* is still unclear. Applying antibiotic usage strategies and improving hygiene is necessary for these locations. Further studies may help understand the interaction of AMR bacteria and resistance genes in all environments.

ACKNOWLEDGMENTS. We are grateful to the staff of the Hanoi Center for Disease Control and Vietnam Research Station of Nagasaki University for their assistance. This work was supported by the Research Program on Emerging and Re-emerging Infectious Diseases (17fk0108122j1901 and 18fk0108049j1902) from the Japan Agency for Medical Research and Development (AMED) (NK), the Japan Initiative for Global Research Network on Infectious Diseases and the Research Program on Emerging and Re-emerging Infectious Diseases (18fm0108001) from AMED (FH), and Mr. Isao Shinjo and Mr. Kentaro Okuno (KM).

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