



# Antimicrobial resistance profiles and molecular characterization of *Escherichia coli* strains isolated from healthy adults in Ho Chi Minh City, Vietnam

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**ABSTRACT.** In this study, we attempted to isolate *Escherichia coli* from healthy adults in Ho Chi Minh City, Vietnam, and characterized its antimicrobial resistance profile, extended-spectrum  $\beta$ -lactamase (ESBL) genotype, phylogenetic grouping and virulence gene profile. A total of 103 *E. coli* isolates were obtained, and most of them were antimicrobial resistant such to streptomycin (80.6%), tetracycline (67.0%), ampicillin (65.0%), sulfamethoxazole/trimethoprim (48.5%), nalidixic acid (43.7%), chloramphenicol (34.0%), cefotaxime (15.5%), ciprofloxacin (15.5%), kanamycin (12.6%), ceftazidime (10.7%), fosfomycin (4.9%) and gentamicin (2.9%). However, all these *E. coli* strains were susceptible to imipenem. Surprisingly, of 103 strains, 74 (71.8%) and 43 (41.7%) strains showed resistance to more than 3 and 5 classes of antimicrobials, respectively. Furthermore, 10 *E. coli* strains were ESBL-producers and positive for *bla*<sub>CTX-M</sub> genes (7 for *bla*<sub>CTX-M-9</sub> and 3 for *bla*<sub>CTX-M-1</sub>), while five were additionally positive for *bla*<sub>TEM</sub> genes. S1-nuclease pulsed-field gel electrophoresis analysis revealed that 7 and 3 strains of *E. coli* carry *bla*<sub>CTX-M</sub> genes on their large plasmid and chromosome, respectively. Phylogenetic analysis exhibited that majority of the *E. coli* strains was grouped into A (44.7%), followed by B1 (23.3%), B2 (18.4%) and D (13.6%). Virulence genes associated with diarrheagenic *E. coli*, such as *astA*, *EAF*, *eaEA*, *elt* and *egg* were also detected in ESBL-producing *E. coli* as well as antimicrobial resistant strains. These data suggest that commensal *E. coli* of healthy human could be a reservoir for antimicrobial resistance determinants and some of them might be harmful to human.

**KEY WORDS:** *E. coli*, ESBL, healthy individual, virulence gene

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Antimicrobial agents have contributed not only in human medicine but also in veterinary medicine and animal-originated food productions. On the other hand, emergence and dissemination of antimicrobial resistant bacteria, particularly multidrug resistance (MDR), has become a serious global concern [20, 35].

*Escherichia coli* are commensal bacteria in the gut of warm-blooded animals including human and constitute a part of the normal flora [13, 18]. However, some of them can cause diseases in human and animals, such as intestinal and extra-intestinal infections [13, 18], including diarrhea, urinary tract infection, meningitis, septicemia and so on. Resistance to antimicrobial agents of both pathogenic and nonpathogenic *E. coli* strains from human, animal and food origin has been reported [37]. Furthermore, due to widespread use of antimicrobial agents in animal husbandry, it may be a selector for resistance, and *E. coli* strains can be rapidly spread among animals and transferred to humans [37]. Humans and animals are probably main reservoir of antimicrobial resistant *E. coli*, and their transmission can occur by direct contact or via food chain [24, 37]. Indeed, several studies found that the acquired antimicrobial resistance genes found in human *E. coli* isolates are closely associated to the genes found in animal isolates [24, 37].

Gut of a healthy individual is a probable reservoir of the microbial population carrying virulence and antimicrobial resistance related genes including the extended spectrum beta-lactamase (ESBL)-producing strains. A number of studies have been described about antimicrobial resistant commensal *E. coli* isolated from not only diseased patients [8, 17, 30, 43] but also healthy children and adults worldwide [2, 8, 14, 32]. Recently, much attention has been paid to ESBL-producing *E. coli* in healthy individuals as well [1, 4, 5, 15, 21, 22, 32, 42, 44, 47, 50]. In Vietnam, prevalence of antimicrobial resistance in commensal *E. coli* was

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investigated in only children [14], raw food samples [49], household and small chicken farms [31]. However, comprehensive studies regarding analysis of antimicrobial resistance, ESBL type and virulence gene profile of commensal *E. coli* in adults were not conducted.

The study of the commensal *E. coli* population from healthy individuals is of great significance to understand and monitor the prevalence, distribution and carriage of pathogenic traits and antimicrobial resistance. The potentially pathogenic microbes with antimicrobial determinants may spread to other individuals not only from human but also animal and animal originated foods. On the other hand, the antimicrobial determinants may be transferred to more pathogenic bacteria in this niche. Keeping these factors in mind, this study was designed to characterize the commensal *E. coli* strains isolated from healthy adults of Ho Chi Minh City, Vietnam, for the presence of virulence gene, antimicrobial resistance profile and ESBL detection.

## MATERIALS AND METHODS

### *Bacterial isolates*

A total of 103 stool specimens were collected from healthy adults whose ages ranged from 20 to 70 years in 3 urban districts (73 adults in 3, 6 and Tan Phu) and 1 suburban district (30 adults in Cu Chi) in Ho Chi Minh City, Vietnam, during March to November 2013. It was confirmed that none of the study participants had history of taking antimicrobials at least 3 months before the sample collection. All people whose samples were collected gave their informed consent for the participation in this study. Stool specimens were inoculated onto CHROMagar Orientation (CHROMagar Paris France, Paris, France). After incubation at 37°C for 22 ± 2 hr, a dark pink to reddish colony was selected and then identified to be *E. coli* by the IMViC tests [19]. One *E. coli* strain was collected from each sample and used for further analysis.

### *Determination of antimicrobial susceptibility*

Antimicrobial susceptibility of the *E. coli* strains against ampicillin (AMP), cefotaxime (CTX), ceftazidime (CAZ), imipenem (IPM), nalidixic acid (NAL), ciprofloxacin (CIP), gentamicin (GEN), kanamycin (KAN), streptomycin (STR), fosfomycin (FOS), tetracycline (TET), trimethoprim/sulfamethoxazole (SXT) and chloramphenicol (CHL) was examined by disk diffusion method [11] on Mueller-Hinton agar (Becton Dickinson and Co., Sparks, NV, U.S.A.) using commercially available disks (Becton Dickinson and Co.). Characterization of *E. coli* strains as susceptible, intermediate resistant or resistant was based on the size of the inhibition zones according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. *E. coli* strain ATCC 29522 was used as a control strain in the susceptibility test. MDR was defined as resistance to at least one antimicrobial agent in three or more classes of antimicrobial agents [6].

### *ESBL phenotype and genotype confirmation*

ESBL phenotype was analyzed by a double-disk diffusion test using CTX, CAZ, CTX/clavulanic acid and CAZ/clavulanic acid [10]. ESBL (*bla*<sub>CTX-M</sub>, *bla*<sub>TEM</sub> and *bla*<sub>SHV</sub>) genes were determined by a multiplex PCR as described previously [27]. Genotype of *bla*<sub>CTX-M</sub> genes was further analyzed by PCR using four primer sets targeting the CTX-M-1, CTX-M-2, CTX-M-8 and CTX-M-9 groups [40].

### *Plasmid profile and location of bla<sub>CTX-M</sub> gene*

Plasmid profiles of ESBL-producing *E. coli* strains were examined by S1-nuclease-PFGE with some modifications [3]. Briefly, a single genomic DNA block was incubated at 37°C for 40 min with 4 units of S1 nuclease (Thermo Fisher Scientific, Waltham, MA, U.S.A.) in 200 µl of 30 mM sodium acetate buffer (pH 4.6) including 50 mM NaCl, 1.0 mM zinc acetate and 5% (v/v) glycerol. The digested DNA was electrophoresed on a 1.0% pulsed-field certified agarose (Bio-Rad Laboratories Inc., Hercules, CA, U.S.A.) in 0.5X TBE (45 mM Tris, 45 mM boric acid and 1 mM EDTA) buffer at 6 V/cm<sup>2</sup>, with a switch time of 2–60 sec for 21 hr using the CHEF Mapper machine (Bio-Rad Laboratories Inc.). *Xba*I-digested *Salmonella* serotype Braenderup H98121 was used as a molecular size marker.

Location of *bla*<sub>CTX-M</sub> genes was analyzed by Southern hybridization. The resolved plasmids and genomic DNA by S1-PFGE gels were transferred onto nylon membranes (Perkin Elmer, Waltham, MA, U.S.A.). The membrane was hybridized with a specific <sup>32</sup>P-labeled DNA probe as described below. The *bla*<sub>CTX-M-1</sub> and *bla*<sub>CTX-M-9</sub> gene-probes were prepared by PCR using primers and conditions as described elsewhere [40] and labeled by random priming method using MultiPrime DNA Labeling System (GE Healthcare UK, Buckinghamshire, U.K.) with [ $\alpha$ -<sup>32</sup>P]-dCTP (111 TBq/mmol) (Perkin Elmer). Radioactivity was visualized by the BAS FLA-3000 system (GE Healthcare, Tokyo, Japan).

### *Phylogenetic characterization*

Phylogenetic group of *E. coli* strains was determined by using a multiplex PCR as reported previously [9].

### *Detection of virulence genes for diarrheagenic E. coli*

A total of 103 *E. coli* strains were analyzed for the presence of virulence genes associated with diarrheagenic *E. coli* (DEC) including *eae* (*E. coli*-attaching and effacing), *bfpA* (bundle-forming pilus), EAF (enteropathogenic *E. coli* adherence factor), *elt* (heat-labile enterotoxin), *est* (heat-stable enterotoxin), *eagg* (plasmid of enteroaggregative *E. coli*), *astA* (enteroaggregative *E. coli* heat-stable enterotoxin 1), *stx1* (Shiga toxin 1), *stx2* (Shiga toxin 2), *invE* and *daaD* by colony hybridization assay using respective

probe labeled with [ $\alpha$ -<sup>32</sup>P]-dCTP (Perkin Elmer) as described previously [36].

## RESULTS

### Antimicrobial susceptibility

A total of 103 *E. coli* strains including 73 from urban districts and 30 from suburban district were successfully isolated from 103 healthy adults in Ho Chi Minh City, Vietnam, and further analyzed for their antimicrobial susceptibility to 13 different drugs including AMP, CTX, CAZ, IPM, NAL, CIP, GEN, KAN, STR, FOS, TET, SXT and CHL. In this study, *E. coli* strains showing intermediate antimicrobial resistance were interpreted as resistant. As shown in Table 1, most of the *E. coli* strains were resistant to STR (80.6%), TET (67.0%), AMP (65.0%), SXT (48.5%) and NAL (43.7%), although all *E. coli* strains were susceptible to IPM. Moreover, 96.1% of the *E. coli* strains (99/103) were resistant to one or more antimicrobial agents. Notably, MDR was detected in 71.8% (74/103) of the tested strains. In addition, extensive MDR, which was defined to be resistant against at least five different classes of antimicrobial agents, was detected in 41.7% (43/103) strains. There was no significant difference in rate of MDR of *E. coli* strains isolated from healthy adults in urban (54/73; 74.0%) and suburban districts (20/30; 66.7%).

### ESBL characterization and its genotype

Since 51, 5 and 11 *E. coli* strains were resistant to AMP alone, AMP and CTX, AMP, CTX and CAZ, respectively, these strains might produce ESBL. Indeed, 10 out of the 67 strains were confirmed to be ESBL-producing *E. coli*. Among 10 ESBL-producing *E. coli* strains, 9 were isolated from healthy adults in urban districts, while 1 was from healthy adult in suburban district. Subsequently, ESBL genes were characterized for these 10 ESBL-producing *E. coli* strains, and the result is summarized in Table 2. The *bla*<sub>CTX-M</sub> genes were detected in all the ESBL-producing *E. coli*. Moreover, five CTX-M-positive *E. coli* strains additionally possessed *bla*<sub>TEM</sub> genes, but none of the strains were positive for the *bla*<sub>SHV</sub> genes. Analysis of genotype for the *bla*<sub>CTX-M</sub> genes

**Table 1.** Antimicrobial resistant *E. coli* strains isolated from healthy adults fecal samples (n=103)

	AMP <sup>a)</sup>	CTX <sup>b)</sup>	CAZ <sup>c)</sup>	IPM <sup>d)</sup>	NAL <sup>e)</sup>	CIP <sup>f)</sup>	GEN <sup>g)</sup>	KAN <sup>h)</sup>	STR <sup>i)</sup>	FOS <sup>j)</sup>	TET <sup>k)</sup>	SXT <sup>l)</sup>	CHL <sup>m)</sup>
No. of isolates	67	16	11	0	45	16	3	13	83	5	69	50	35
%	65.0	15.5	10.7	0.0	43.7	15.5	2.9	12.6	80.6	4.9	67.0	48.5	34.0

a) ampicillin, b) cefotaxime, c) ceftazidime, d) imipenem, e) nalidixic acid, f) ciprofloxacin, g) gentamicin, h) kanamycin, i) streptomycin, j) fosfomycin, k) tetracycline, l) trimethoprim-sulphamethoxazole, m) chloramphenicol.

**Table 2.** Characteristics of *E. coli* strains, isolated from the fecal samples of healthy adults, carrying ESBL and/or virulence genes

Isolate ID	Place	Phylogenetic group	Virulence gene <sup>a)</sup>	Antimicrobial resistance phenotype	$\beta$ -lactamase genes	Location of <i>bla</i> <sub>CTX-M</sub>
E33	District 3	A	ND <sup>b)</sup>	AMP-CTX-CAZ	<i>bla</i> <sub>CTX-M-1</sub> , <i>bla</i> <sub>TEM</sub>	Plasmid
E42	Tan Phu	A	<i>astA</i>	AMP-CTX-NAL-TET-SXT	<i>bla</i> <sub>CTX-M-9</sub> , <i>bla</i> <sub>TEM</sub>	Chromosome
E55	Tan Phu	D	<i>astA</i>	AMP-CTX-NAL-SXT-STR	<i>bla</i> <sub>CTX-M-9</sub> , <i>bla</i> <sub>TEM</sub>	Plasmid
E66	Tan Phu	A	ND	AMP-CTX-CAZ-NAL-TET-SXT-STR-CHL	<i>bla</i> <sub>CTX-M-1</sub> , <i>bla</i> <sub>TEM</sub>	Plasmid
E95	District 6	B1	ND	AMP-CTX-CAZ-NAL-TET-SXT-CIP	<i>bla</i> <sub>CTX-M-1</sub> , <i>bla</i> <sub>TEM</sub>	Chromosome
E96	District 6	D	ND	AMP-CTX-NAL-TET-SXT-STR-CHL-CIP	<i>bla</i> <sub>CTX-M-9</sub>	Chromosome
E97	District 6	B1	<i>astA</i>	AMP-CTX-NAL-KAN	<i>bla</i> <sub>CTX-M-9</sub>	Plasmid
E100	District 6	B1	<i>astA</i> , EAF	AMP-CTX-NAL-KAN	<i>bla</i> <sub>CTX-M-9</sub>	Plasmid
E101	District 6	B1	<i>astA</i> , EAF	AMP-CTX-NAL-KAN	<i>bla</i> <sub>CTX-M-9</sub>	Plasmid
E137	Cu Chi	B2	ND	AMP-CTX-NAL-TET-SXT-STR-CIP	<i>bla</i> <sub>CTX-M-9</sub>	Plasmid
E21	District 3	A	<i>astA</i>	NAL	NA <sup>c)</sup>	NA
E23.13	District 3	B1	<i>astA</i>	AMP-CHL-SXT	NA	NA
E25.13	District 3	A	<i>astA</i>	AMP-CHL-CIP-FOS-GEN-KAN-NAL-STR-TET-SXT	NA	NA
E35.13	Tan Phu	A	<i>astA</i> , <i>elt</i>	CHL-KAN-STR-TET-SXT	NA	NA
E41.13	Tan Phu	D	<i>astA</i> , <i>eaeA</i>	AMP-CTX-CAZ-CIP-NAL-STR-TET	NA	NA
E48.13	Tan Phu	B1	EAF, <i>eagg</i>	AMP-STR-TET	NA	NA
E62.13	Tan Phu	A	<i>astA</i>	FOS-STR	NA	NA
E63.13	Tan Phu	A	<i>astA</i> , <i>eagg</i>	AMP-STR-TET-SXT	NA	NA
E64.13	Tan Phu	A	EAF	AMP-CHL-FOS-STR-TET-SXT	NA	NA
E65.13	Tan Phu	A	EAF	KAN-NAL-TET	NA	NA
E135.13	Cu Chi	B1	<i>astA</i> , <i>elt</i>	STR	NA	NA
E136.13	District 3	D	<i>astA</i> , <i>elt</i>	AMP-CHL-STR-TET-SXT	NA	NA
E153.13	Cu Chi	B1	<i>astA</i>	AMP-CHL-CIP-KAN-NAL-STR-TET-SXT	NA	NA

a) *eaeA* (*E. coli*-attaching and effacing), EAF (enteropathogenic *E. coli* adherence factor), *elt* (heat-labile enterotoxin), *eagg* (plasmid of enteroaggregative *E. coli*), *astA* (enteroaggregative stable enterotoxin). b) not detected, c) not applicable.

exhibited that most of the *E. coli* strains belonged to *bla*<sub>CTX-M-9</sub> group (70.0%), followed by *bla*<sub>CTX-M-1</sub> group (30.0%).

#### Plasmid profiles and location of *bla*<sub>CTX-M</sub> genes

In general, the ESBL genes are predominantly located on plasmids and are easily disseminated. To see if ESBL genes are located on plasmid or chromosome, plasmid profile and location of *bla*<sub>CTX-M</sub> genes were analyzed for the 10 ESBL-producing *E. coli* strains by PFGE with S1-nuclease digestion and Southern hybridization. As shown in Fig. 1, there was at least one large plasmid ranging from 50 to 244 kb in size and a variation of plasmid profiles in ESBL-producing *E. coli*.

The Southern hybridization of S1-PFGE gel demonstrated that most of the ESBL-producing *E. coli* (7/10 isolates) harbored the *bla*<sub>CTX-M</sub> genes on a large plasmid (ranging from 50 to 200 kb in size) as shown in Fig. 1. Other 3 strains, however, carried the *bla*<sub>CTX-M</sub> genes on chromosome. Moreover, among the 5 strains harboring both *bla*<sub>CTX-M</sub> and *bla*<sub>TEM</sub> genes, 3 strains carried these genes on the same plasmid (data not shown).

#### Phylogenetic characterization

We further examined the 103 *E. coli* strains for phylogenetic group. The majority of the *E. coli* strains isolated from healthy humans belonged to phylogenetic group A (44.7%) followed by B1 (23.3%), B2 (18.4%) and D (13.6%) groups (data not shown). Moreover, the highest prevalence of *E. coli* strains showing MDR was distributed into the phylogenetic group A (34/74, 45.9%), followed by groups B1 (16/74, 21.6%), B2 (13/74, 17.6%) and D (11/74, 14.9%). Most ESBL-producing *E. coli* strains belonged to phylogenetic groups A (3/10, 30%) and B1 (4/10; 40%).

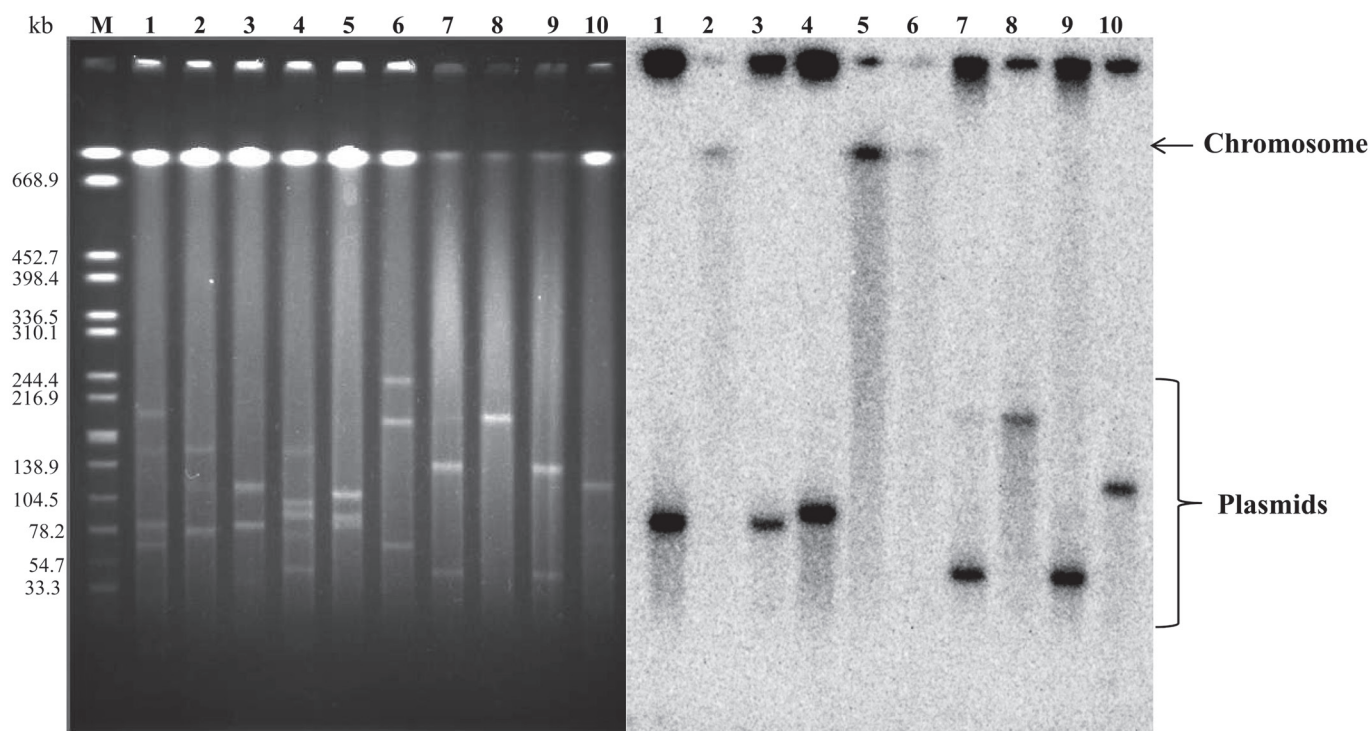
#### Virulence gene profile

Distribution of virulence genes associated with DEC was examined, and the result is shown in Table 2. Of 103 *E. coli* strains, none of the strains were positive for *bfpA*, *est*, *stx1*, *stx2*, *invE* and *daaD* genes. However, *astA* was most prevalent and detected in 15 strains, followed by EAF (n=5), *elt* (n=3), *eagg* (n=2) and *eae* (n=1), indicating that some of the *E. coli* strains isolated from healthy adults are potentially virulent to human. In addition, 7 *astA* genes were detected in combination with EAF (n=2), *elt* (n=3), *eagg* (n=1) and *eaeA* (n=1). Fifteen out of 18 *E. coli* strains harboring virulence genes were MDR. Thus, the strains carrying both virulence and antimicrobial resistance genes may pose a serious threat to public health. Besides, 8, 7 and 3 *E. coli* strains harboring virulence genes belonged to the phylogenetic groups A, B1 and D, respectively (Table 2). Furthermore, 2 out of these 18 *E. coli* strains harboring virulence genes were isolated from healthy adults in suburban districts.

## DISCUSSION

Emergence of antimicrobial resistant bacteria has been increasingly recognized as a serious problem in the world. Recently, a number of studies regarding prevalence of antimicrobial resistant *E. coli* including ESBL producer in not only hospitalized patients but also healthy people have been reported in many parts of the world [1, 4, 15, 21–23, 32, 42, 47]. However, there was less report regarding the comprehensive analysis of antimicrobial resistant *E. coli* strains isolated from healthy children in Vietnam [14].

In the present study, therefore, we attempted to isolate *E. coli* from healthy adults in Ho Chi Minh City in Vietnam for further characterization of their antimicrobial resistance, ESBL genotype and its location, phylogenetic groups and virulence gene profiles. A total of 103 *E. coli* were successfully isolated, and most of the strains showed resistance to one or more antimicrobials. The results of antimicrobial susceptibility in this study are in accordance with a previous study of healthy children in FilaBavi, near Hanoi, Vietnam, except for CIP [14] in which only 6 antimicrobials, such as AMP, CIP, CHL, NAL, TET, and SXT were examined and the isolated *E. coli* strains were resistant to AMP (65%), TET (74%), CHL (40%), NAL (27%) and SXT (68%). However, <1% of the *E. coli* strains isolated in FilaBavi were resistant to CIP [14], although 15.5% strains were resistant to CIP in this study. Surprisingly, 72% of the *E. coli* strains were MDR, and furthermore, 42% of the *E. coli* strains were extensive MDR. This high rate of MDR was observed in the *E. coli* strains isolated from healthy adults in both urban and suburban districts in Ho Chi Minh City. These percentages are quite high in comparison to those observed in a French study [32], but consistent with those reported from Korea and Bolivia [2, 8]. These results revealed a high risk of fecal carriage of antimicrobial resistant *E. coli* strains in healthy adults in Ho Chi Minh City, Vietnam. One reason could be due to improper use of antimicrobials for human in Vietnam where antimicrobials can be purchased over the counter without a prescription and there is no sanction for non-compliance of drug law regulations [28]. Alternatively, it could be due to improper use of antimicrobials for food-animals [7, 48] and veterinary medicine. The phenomenon of over usage of antimicrobials and thus emergence of MDR is an emerging problem worldwide, especially in developing countries. It could be also possible that antimicrobial resistant *E. coli* might be transmitted from food-animals and raw foods to human. The previous studies from southern Vietnam suggested that food-animals and raw foods could be a potential reservoir of antimicrobial resistance bacteria that disseminated to human [31, 49]. It was found that commensal *E. coli* isolates on household and small-scale chicken farms in the Mekong Delta of Vietnam have high prevalence of resistance to TET, AMP, SXT, and CHL (>60%) and MDR (81.3%), indicating the high risk of transmission of antimicrobial-resistant *E. coli* to community [31]. It was also reported that 61.6% of *E. coli* isolates from raw foods in the marketplace in Ho Chi Minh City were MDR and some of the tested strains contained a pool of mobile genetic elements for dissemination of antimicrobial resistant genes to human [49]. The occurrence rate of ESBL-producing *E. coli* in this study was lower than that of community from Hanoi, Vietnam (46.7%) [46], Thailand (65.7%) [23] and China (50.5%) [21], but consistent with that of community in Libya (13.4%) [1], Tunisia (7.3%) [4], Japan (6.4%) [22], Germany (6.3%) [47], France (6.0%) [32], Netherlands (7.8%) [42] and Switzerland (5.8%) [15]. Higher



**Fig. 1.** Location of the *bla* genes in ESBL-producing *E. coli* strains isolated from fecal samples of healthy humans. Left panel; Separation of S1 nuclease-digested DNAs by PFGE. Right panel; Southern hybridization with a *bla*<sub>CTX-M</sub> gene-probe. Lanes: 1, *E. coli* strain E33; 2, E42; 3, E55; 4, E66; 5, E95; 6, E96; 7, E97; 8, E100; 9, E101; 10, E137. M, *Xba*I-digested DNA from *S. enterica* serotype Braenderup strain H9812 used as molecular size markers.

prevalence of ESBL-producing *E. coli* in Hanoi, Vietnam, and other countries could be due to differences in the isolation protocols. In those studies,  $\beta$ -lactam supplemented agar media were used for the isolation of the *E. coli*. ESBLs can be broadly divided into three groups, such as TEM, SHV and CTX-M, and CTX-M  $\beta$ -lactamases are the most widespread and frequently detected in *E. coli* [39]. In addition, genotype of CTX-Ms was found to be different and specific between regions in the world [50]. In this study, *bla*<sub>CTX-M-9</sub> was found to be the most predominant group in healthy adults in Ho Chi Minh City, which is similar in northern Vietnam [5], Thailand [23] and China [21]. Whereas *bla*<sub>CTX-M-1</sub> group was the most predominant  $\beta$ -lactamase gene in ESBL-producing *E. coli* in healthy carriage in European countries, such as Germany, France, Netherland and Switzerland [15, 32, 42, 47]. In Ho Chi Minh City, it has been reported that *bla*<sub>CTX-M-9</sub> and *bla*<sub>CTX-M-1</sub> groups were the two most predominant types among ESBL-producing *E. coli* strains from foods [29] and patients with nosocomial infections in hospitals [45]. These data suggest that *bla*<sub>CTX-M-9</sub> and *bla*<sub>CTX-M-1</sub> groups might be circulating among foods, healthy adults and clinical settings in Ho Chi Minh City, Vietnam.

It is well known that plasmid plays an important role in the horizontal transfer of antimicrobial resistant genes in microbes including ESBL [12, 25]. In this study, *bla*<sub>CTX-M</sub> genes were detected on plasmids, whose sizes are ranged from about 50 to 200 kb, in 7 out of 10 ESBL-producing *E. coli* strains. Our data indicate that ESBL-producing *E. coli* in healthy carriage could be a potential reservoir and source of dissemination of plasmid-mediated ESBL genes.

Phylogenetic group of *E. coli* strains isolated from healthy adults was investigated. It has been reported that the intestinal pathogenic *E. coli* are mostly classified into phylogenetic groups A, B1 and D [41], whereas strains belonging to phylogenetic group B2 are highly virulent and associated with extra-intestinal infections [38]. Most of the *E. coli* strains isolated in this study were identified to be phylogenetic group A followed by groups B1, B2 and D. This result is consistent with many of the previous reports that *E. coli* from community mainly belonged to phylogenetic group A [9, 47]. Notably, the majority of MDR *E. coli* strains in this study belonged to phylogenetic group A (34/74 strains). Furthermore, 13 of the MDR *E. coli* strains belonged to phylogenetic group B2, suggesting that these strains might have a potential virulence capacity and could pose a risk to public health.

Furthermore, virulence gene profile analysis indicated that indeed 18 *E. coli* strains carried one or two virulence genes associated with DEC (Table 2). Among 11 virulence genes tested, *astA* was most prevalent, and some of the strains carried *eae*, *EAF*, *elt* and *eagg* in addition to *astA*. *astA* encoding EAST1 was initially detected in enteroaggregative *E. coli* (EAEC) [26] and thought to be a specific virulence factor of EAEC. However, *astA* has been detected in *E. coli* strains belonging to various pathotypes [41], and significance of EAST1 in diarrhea is not still clear. On the other hand, there are several reports regarding association of *E. coli* containing only *astA* among various virulence genes for DEC with a few outbreaks all over the world [16, 33, 34, 51], indicating

MDR *E. coli* having DEC-associated virulence genes, in particular *astA*-positive MDR *E. coli* strains, might be harmful to human.

In conclusion, most of the *E. coli* strains isolated from healthy adults in Ho Chi Minh City, Vietnam, are MDR including extensive MDR. Some of them have virulence genes associated with DEC. Together with phylogenetic analysis, these data suggest that *E. coli* strains in healthy adults in southern Vietnam could be reservoir of antimicrobial resistance genes including ESBL and might be harmful to human.

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