Abundance of colistin-resistant *Escherichia coli* harbouring *mcr-1* and extended-spectrum β -lactamase-producing *E. coli* co-harbours *bla*_{CTX-M-55} or *bla*_{TEM} isolates from chicken meat in Vietnam

Tatsuya Nakayama (✉ t-nakayama@hiroshima-u.ac.jp)

Hiroshima University: Hiroshima Daigaku <https://orcid.org/0000-0002-5620-1712>

Le Thi Hien

Ho Chi Minh City Institute of Public Health

Ngo Thanh Phong

Ho Chi Minh City Institute of Public Health

Doan Nguyen Minh Tran

Ho Chi Minh City Institute of Public Health

Oanh Thi Hoang Nguyen

Ho Chi Minh City Institute of Public Health

Hoang Hoai Phuong

Ho Chi Minh City Institute of Public Health

Takahiro Yamaguchi

Osaka Institute of Public Health

Michio Jinnai

Kanagawa-ken Eisei Kenkyujo

Phuc Do Nguyen

Ho Chi Minh City Institute of Public Health

Chinh Van Dang

Ho Chi Minh City Institute of Public Health

Yuko Kumeda

Osaka prefecture University

Atsushi Hase

Tezukayama Daigaku - Higashiikoma Campus: Tezukayama Daigaku

Research Article

Keywords: *mcr-1*, *bla*_{CTX-M-55}, *bla*_{CTX-M-65}, *bla*_{CMY-2}, *bla*_{TEM}, plasmid-mediated antibiotic-resistant *E. coli*

Posted Date: September 20th, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-904323/v1>

License: © ⓘ This work is licensed under a Creative Commons Attribution 4.0 International License. [Read Full License](#)

Version of Record: A version of this preprint was published at Archives of Microbiology on January 15th, 2022. See the published version at <https://doi.org/10.1007/s00203-021-02746-0>.

Abstract

Although the spread of plasmid-mediated antibiotic-resistant bacteria is a public health concern, food contamination with plasmid-mediated antibiotic-resistant *Escherichia coli* has not been well investigated in Vietnam. The aim of this study was to describe the prevalence of colistin-resistant, carbapenem-resistant and endemic *bla*_{CTX-M} in extended-spectrum β -lactamase (ESBL)-producing *E. coli* isolates. Colistin- and carbapenem-resistant ESBL-producing *E. coli* were isolated from chickens in Vietnam and Japan. The results showed that 52% and 93% of Vietnamese chicken was isolated with colistin-resistant and AmpC/ESBL-producing *E. coli*, respectively, while 52.7% of Japanese chickens were isolated with AmpC/ESBL-producing *E. coli*. Carbapenem-resistant *E. coli* has not been isolated in Vietnam or Japan. Genotyping revealed that colistin-resistant *E. coli* harboured *mcr-1*, and most of the AmpC/ESBL-related genes were *bla*_{CTX-M-55} and *bla*_{CTX-M-65} together with *bla*_{TEM} in Vietnamese chickens, and *bla*_{CMY-2} in Japanese chickens. Multidrug resistance analysis showed that ESBL-producing *E. coli* isolates were more resistant to quinolones, streptomycin, and chloramphenicol compared with colistin-resistant *E. coli* isolates from Vietnam, suggesting selection in ESBL-producing *E. coli* for multiple antibiotic resistance genes. In conclusion, colistin-resistant *E. coli* was detected in about half of the chicken meat samples, the majority of which were found to harbour *mcr-1*. The high prevalence of ESBL-producing *E. coli* has remained constant across the last five years, and the predominant *bla*_{CTX-M} for ESBL-producing *E. coli* was found to be *bla*_{CTX-M-55} or *bla*_{CTX-M-65}, with the coexistence of *bla*_{TEM} in Vietnam. Our results can be implemented in monitoring systems to combat the development of antimicrobial resistance.

Introduction

The spread of antibiotic-resistant bacteria is a worldwide public health concern (Giurazza et al. 2021). Plasmid-mediated antibiotic resistance, a critical issue in the study of antibiotic resistance, requires urgent attention due to the development of resistance by horizontal gene transmission (Bevan et al. 2017).

In 2015, there was a report on the acquisition of colistin resistance by a new plasmid-encoded *mcr* gene (Liu et al. 2016). Colistin is expected to be a therapeutic agent for carbapenem-resistant bacteria. Therefore, the spread of colistin-resistant bacteria in society is of great concern. In our study, we found that colistin is frequently used on agricultural and livestock farms in Vietnam (Nakayama et al. 2017). In addition, Yamamoto et al. (2019) found that approximately 70% of Vietnamese local residents carried colistin-resistant bacteria. Although it is assumed that Vietnamese food can lead to human colonisation with colistin-resistant bacteria, pathways of contamination are not clear. This is especially true in chickens, where plasmid-mediated drug-resistant bacteria are frequently isolated.

ESBL and carbapenem-resistant bacteria are often associated with plasmid-mediated antibiotic resistance. Recent studies have focused on ESBL-producing *Escherichia coli*, a bacterium demonstrating plasmid-mediated antibiotic resistance (Bevan et al. 2017). Current knowledge suggests that ESBL-producing bacteria in food and humans is detected more frequently in Vietnam than in Japan (Nakayama et al. 2015; Le et al. 2015). In an earlier study in Vietnam, we found that ESBL-producing *E. coli* were frequently detected in chicken meat between 2013–2016. Most of the ESBL-producing *E. coli* isolates carried ESBL-related genes in the *bla*_{CTX-M-1} and *bla*_{M-9} groups (Nakayama et al. 2015).

Monitoring of antibiotic-resistant bacteria is currently underway in many countries around the world. Therefore, it is very important to identify not only the presence of ESBL-producing *E. coli* but also the *bla*_{CTX-M} genotype, which is currently widespread. Carbapenem-resistant *E. coli* are also well known as plasmid-mediated antibiotic-resistant *E. coli*, but research is lacking with regards to its importance and the degree of contamination in chicken. Hence, as with ESBL-producing *E. coli*, caution is required for the spread of carbapenem-resistant *E. coli*. In Japan, food contamination with AmpC/ESBL-producing *E. coli* was found in multiple studies (Kameyama et al. 2013; Hiroi et al. 2012). Reports indicate that *bla*_{CMY-2} and *bla*_{CTX-M-2} are the most abundant ESBL-producing *E. coli* in chickens. There are no reports of carbapenem-resistant and colistin-resistant *E. coli* isolated from chickens in Vietnam or Japan. Therefore, the aim of this study was to determine and compare the prevalence of colistin, carbapenem-resistant, and ESBL-producing *E. coli* in chickens of Vietnam and Japan.

Materials And Methods

Sampling locations and bacterial isolation

A total of 134 chicken samples (including 60 meat samples were purchased in retails or supermarkets, Ho Chi Minh City, Vietnam, and 33 meat samples were purchased in supermarkets and 41 faecal samples from laying hens in Japan) were investigated. Chicken meat (25 g) was mixed with 225 mL of buffered peptone water (BPW) or 1 g of faeces was mixed with 9 mL of BPW. After gentle shaking, 100 μ L of the solution was spread on MacConkey agar (Eiken Chemical, Tochigi, Japan) containing 2 μ g mL⁻¹ of cefotaxime (CTX) for AmpC/ESBL-producing *E. coli* isolation, 2 μ g mL⁻¹ colistin for colistin-resistant *E. coli* isolation, or 0.25 μ g mL⁻¹ of meropenem (MEM) for carbapenem-resistant *E. coli* isolation, and finally incubated at 37 °C for 21 $\pm$ 3 h. After incubation, one to three colonies were picked for further analysis.

Bacterial identification and DNA extraction

Isolated bacteria were confirmed as *E. coli* by biochemical tests, such as triple sugar iron (Eiken Chemical, Tokyo, Japan) and lysin indole motility (Eiken Chemical, Tochigi, Japan). Bacterial DNA was extracted by boiling suspensions of the isolates in Tris (hydroxymethyl) aminomethane-EDTA

buffer (Nakayama et al. 2015). Isolated bacteria were also identified as *E. coli* using ECN amplification (Hoa et al 2020).

Multiplex PCR for phylogenetic group

The phylogenetic groups of isolated bacteria were determined by multiplex PCR amplification of a combination of two genes, *chuA* and *yjaA*, and a DNA fragment, TspE4C2 (Le et al. 2015). The extracted DNA was amplified using a multiplex PCR kit (Qiagen, Hilden, Germany). Primer sets for multiplex PCR are specified in Table S1. PCR was conducted under the following conditions: 35 cycles of denaturation at 98 °C for 10 s, annealing at 57 °C for 30 s, and extension at 72 °C for 30 s. The PCR amplified products were visualized using 1.5% agarose gel and stained by midori-green (Nippon genetics, Tokyo, Japan).

Antibiotic susceptibility testing

After subculturing, the isolated bacteria were suspended in Mueller Hinton (MH) broth (BD, Franklin Lakes, USA). The bacterial suspension was adjusted at MacFarland 0.5, and then the bacterial solution was spread on MH agar (BD, Franklin Lakes, USA) using a sterile cotton swab. *E. coli* isolates were tested for susceptibility to 14 antimicrobial agents: tetracycline (TET), ampicillin (AMP), nalidixic acid (NAL), kanamycin (KAN), chloramphenicol (CHL), streptomycin (STR), trimethoprim/sulfamethoxazole (SXT), gentamicin (GEN), ciprofloxacin (CIP), CTX, ceftazidime (CAZ), meropenem (MEM), and ceftiofur (CFX) (BD Japan, Tokyo, Japan). The disc diffusion method was subsequently used to measure the inhibition zone after bacterial incubation at 37 °C for 21 h, following the standard procedure of the Clinical Laboratory Standards Institute (CLSI 2017). The ESBL phenotype was determined by the double-disk synergy test, using ceftazidime and CTX with and without clavulanic acid (BD, Franklin Lakes, USA), following the guidelines of the CLSI (2017). The AmpC phenotype was also determined using the ceftiofur disc, and MEM resistance was evaluated as carbapenem resistance.

Multiplex PCR for *mcr* and AmpC/ESBL-related genes

The genotype of AmpC/ESBL-producing *E. coli* was determined using multiplex PCR, as described previously (Le et al. 2015; Perez-Perez et al. 2002). Extracted DNA was amplified using a multiplex PCR kit (Qiagen, Hilden, Germany). Multiplex PCRs were conducted under the following conditions: for ESBL, 25 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 90 s, and extension at 72 °C for 90 s; for AmpC, 25 cycles of denaturation at 95 °C for 30 s, annealing at 64 °C for 90 s, and extension at 72 °C for 60 s. Primers for multiplex PCR of both AmpC and ESBL-related genes are described in Table S1. The PCR amplified products were visualized using 3% agarose gel and stained by midori-green (Nippon genetics, Tokyo, Japan).

Identification *bla*_{CTX-M} and *bla*_{CMY-2}

After determining the ESBL genotypes, the results from both the *bla*_{CTX-M-1}, *bla*_{CTX-M-2}, and *bla*_{CTX-M-9} groups were sequenced. We followed the protocol as described in previous studies (Chanawong et al. 2002; Imoto et al. 2014; Harada et al. 2017; Hoang et al. 2017). In the *bla*_{CTX-M-1} group, the *bla*_{CTX-M-1} sequence was amplified using the primer sets ctx-m15-168F and ctx-m3-1059R. The amplified gene was sequenced using the primer sets ctxm1-185F and ctxm3-492R. For the *bla*_{CTX-M-2} and *bla*_{CTX-M-9} groups, the gene was amplified using the primer sets ctx-m2s-1f and ctx-m2-869f, and toho2-48F and ctxm14-903R, respectively. The amplified gene was sequenced using the primer sets toho1-1R, ctx-m2-316f, toho2-411F and ctxm9-490R, respectively. To identify *bla*_{CMY-2}, the *bla*_{CMY-2} gene was amplified using cmv-F and cmv-R primers, and the amplified gene was sequenced using CMY2-outF and CMY2-outR. Primer information is described in Table S1. Each assembled gene sequence was identified using a BLAST search (<https://blast.ncbi.nlm.nih.gov/Blast.cgi>).

Results

Prevalence of chicken contamination with *E. coli* harbouring *mcr*, AmpC/ESBL, and carbapenemase-related genes

In a study of 60 samples of chicken meat from Vietnam, AmpC/ESBL-producing *E. coli* was isolated from 90% (54/60) of the samples, and 52% (26/50) of the chicken meat samples were found to have colistin-resistant *E. coli*, whereas carbapenem-resistant *E. coli* was not detected (Table 1). In comparison, AmpC/ESBL-producing *E. coli* was isolated from 36.4% (12/33) of Japanese chicken meat. Neither colistin- or carbapenem-resistant *E. coli* were detected (Table 1).

Table 1
Prevalence of *E. coli* harbouring *mcr*, AmpC/ESBL, and carbapenemase-related genes in chickens.

Location	Number of chicken samples	Number of chicken samples contamination with <i>E. coli</i> harbouring antibiotic resistance genes			Number of colistin-resistant <i>E. coli</i> isolates and tested	Number of <i>E. coli</i> harbouring antibiotic resistance genes	Number of CTX-resistant <i>E. coli</i> isolates	Number of CTX-resistant <i>E. coli</i> tested	Number of <i>E. coli</i> harbouring antibiotic resistance genes
		<i>mcr</i> gene	AmpC/ESBL-related gene	carbapenemase-related gene					AmpC/ESBL-related genes
Vietnam	60 (chicken meat)	43.3% (26/60)	90% (54/60)	0	36	35	166	152	135
Japan	33 (chicken meat)	0	36.4% (12/33)	0	0	0	23	14	14
	41 (faeces in laying hens)	0	65.9% (27/41)	0	0	0	70	43	43
	Total 74	0	52.7% (39/74)	0	0	0	93	57	57
* Significant difference between chicken meat from Vietnam and Japan (p < 0.01)									

Phylogenetic group of *E. coli*

Among AmpC/ESBL-producing *E. coli* isolated from Vietnamese chicken, type B2 was the least common (7.7%), while other types showed similar results (21.1–26.3%). Type B2 was not detected in colistin-resistant *E. coli*. Other types showed similar results to ESBL-producing *E. coli* (22.9–25.9%) (Table 2). In Japanese chickens, high rates of B1 (43.9%) and D (31.6%) types of ESBL-producing *E. coli* were isolated. Type B2 (3.5%) had a low detection rate, similar to the *E. coli* strains in Vietnamese chicken (Table 2).

Table 2
Phylogenetic grouping of *E. coli* isolates.

	Vietnam	Japan
	Number of <i>E. coli</i> harboring <i>mcr</i> gene	Number of AmpC/ESBL-producing <i>E. coli</i>
A	25.9% (9/35)	21.1% (32/152) 10.5% (6/57)
B1	25.9% (9/35)	25.7% (39/152) 43.9% (25/57)*
B2	0	7.7% (4/152) 3.5% (2/57)
D	22.9% (8/35)	26.3% (40/152) 31.6% (18/57)
NT	25.9% (9/35)	24.3% (37/152) 10.5% (6/57)*

E. coli harbouring *mcr* gene

Isolated *E. coli* was used to detect *mcr* using multiplex primers. The results showed that one strain was undetectable and *mcr-1* was detected in all of the remaining *E. coli* isolates (Table 3).

Table 3
Colistin-resistant *E. coli* harbouring *mcr-1*.

	Number of <i>E. coli</i>
Isolated colistin-resistant <i>E. coli</i>	36
<i>mcr-1</i>	35
<i>mcr-2</i>	0
<i>mcr-3</i>	0
<i>mcr-4</i>	0
<i>mcr-5</i>	0
non-detection	1

ESBL-producing *E. coli* co-harbouring *bla*_{CTX-M} and *bla*_{TEM}

In 115 ESBL-producing *E. coli* isolates from Vietnamese chickens, *bla*_{CTX-M-1} (67%) tended to be more prevalent than the *bla*_{CTX-M-9} (28.7%) group, which was also the case in the 39 Japanese isolates (*bla*_{CTX-M-1} = 53.8%, *bla*_{CTX-M-9} = 23.1%). The results of *bla*_{CTX-M} identification showed that *bla*_{CTX-M-55} (58.3% [67/115]) of the *bla*_{CTX-M-1} group was the most common *bla*_{CTX-M} in Vietnamese samples, followed by *bla*_{CTX-M-65} (18.2% [21/115]) of the *bla*_{CTX-M-9} group (Fig. 1a). *bla*_{CTX-M-1} (23.1% [9/39]), of the *bla*_{CTX-M-1} group, and *bla*_{CMY-2} (23.1% [9/39]) were found to be the most common in Japanese samples, followed by *bla*_{CTX-M-2} of the *bla*_{CTX-M-2} group (12.8% [5/39]) (Fig. 1b). Multiple ESBL-related genes were detected in Vietnamese samples. In particular, the combination of *bla*_{CTX-M} and *bla*_{TEM} was found to be present in more than 70% of the strains (Table 4).

Table 4
E. coli co-harbours *bla*_{CTX-M} and *bla*_{TEM} genes in Vietnam.

	CTX-M Sub-group	CTX-M or CMY-2	Number of AmpC/ESBL-producing <i>E. coli</i>	
			Vietnam	Japan
ESBL-related genes	CTX-M-1 group	M-1		9
		M-15	2	1
		M-55	22	
		M-1/TEM	2	7
		M-15/TEM	3	3
		M-15/TEM/SHV		1
		M-55/TEM	45	
		M-244/TEM	1	
		M-79/TEM	1	
		M-114/TEM	1	
			CTX-M 1 group	67% (77/115)
	CTX-M-9 group	M-14	2	4
		M-27		2
		M-65	4	
		M-125	1	
		M-14/TEM	4	1
		M-24/TEM	1	
		M-27/TEM	2	2
		M-65/TEM	17	
		M-125/TEM	1	
		M-129/TEM	1	
			CTX-M-9 group	28.7% (33/115)
	CTX-M-1 and 9 group	M-1, 65	1	
		M-1,129/TEM	1	
		M-55, 65/TEM	3	
	CTX-M-2 group	M-2		5
		CTX-M-2/TEM		2
	SHV group	SHV		2
		CTX-M group with TEM		72.2% (83/115)*
AmpC type beta-lactamse related genes	CIT group	CMY-2	13	16
		CMY-2/TEM	2	
		Other CITg	1	
		Other CITg/TEM	2	
		CIT group	15.7% (18/115)	13.9% (16/115)
	CIT group with TEM		22.2% (4/18)	0
	*Significance difference between ESBL-producing <i>E. coli</i> harbouring CTX-M group with TEM isolates in Vietnam and Japan (p < 0.01)			
a ESBL-producing <i>E. coli</i> strains in Vietnam.				

Antibiotic susceptibility and multidrug-resistant *E. coli*

The susceptibility test results showed that colistin-resistant *E. coli* harbouring *mcr* differed significantly from the Vietnamese chicken-derived ESBL strains in third-generation cephalosporins and quinolones (Fig. 2a, 2b), and the result of multidrug resistance showed that the highest numbers of drug resistance were 4 and 6, with an average of 6.7 (Fig. 3).

Compared to strains in Japanese chickens, ESBL-producing *E. coli* isolates from Vietnamese chickens had a significantly higher percentage of resistance to all treatments except beta-lactams (Fig. 2b, 2c). The percentage of resistance to quinolones, SXT, and chloramphenicol was particularly high in ESBL-producing *E. coli* isolates from Vietnam. In multidrug resistance, ESBL-producing *E. coli* showed the highest number of drug-resistant strains (8) (average number of drug resistant strains was 9.1), followed by 11 drug-resistant strains. In comparison, in ESBL-producing *E. coli* isolates from Japanese chickens, 4 and 3 kinds of drug resistance were the most common, with an average drug resistance of 4.7 (Fig. 3).

Discussion

The contamination of Vietnamese food with colistin-resistant bacteria has not been sufficiently studied. Nguyen et al (2021) investigated colistin-resistant *E. coli* isolated from 15 chickens and found that colistin resistance was found in 66.7% of the sample group. In their analysis of chicken faeces from backyard chickens in Vietnam, Kawahara et al. (2019) found that 97.2% of the chickens carried *E. coli* harbouring the *mcr* gene. Le et al. (2021) investigated colistin resistance in ESBL-producing *E. coli* isolated from chickens. They found that 53.2% of ESBL-producing *E. coli* isolates were colistin-resistant, and all of these colistin-resistant isolates harboured *mcr-1* (Le et al. 2021). In this study, 43.3% of the food products contained colistin-resistant *E. coli* and almost all of which harboured *mcr-1*, suggesting that *mcr-1* may be spreading between chickens in Vietnam.

To easily isolate colistin-resistant *E. coli*, we analysed chicken faeces from Japan but no trace of the bacteria was found. Therefore, colistin-resistant *E. coli* did not expand in the Japanese chickens. In this study, carbapenem-resistant *E. coli* was not isolated from either Vietnamese or Japanese chickens. Although it can be isolated in hospitals and the environment (Mathys et al. 2019; Zhong et al. 2021), carbapenem-resistant *E. coli* is not generally transmitted to foods in Vietnam or Japan.

In this study, the phylogenetic group was determined for colistin-resistant and ESBL-producing *E. coli* isolates. Colistin-resistant *E. coli* showed a similar pattern to that of ESBL-producing Vietnamese strains. Prevalence of B2 type *E. coli* was much lower compared to the other types. Although no study has investigated the pattern of phylogenetic grouping, normal *E. coli* isolates from chickens, it is possible that the absolute number of *E. coli* type B2 bacteria contamination is low. The phylogenetic group of ESBL-producing *E. coli* in Japanese strains showed that the B1 and D types were very high. This result may be due to regional differences.

As monitoring data to combat antibiotic resistance, it is necessary to clarify the actual condition of antibiotic resistance genes in chicken meat that are frequently contaminated. In Vietnam, the *bla*_{CTX-M} gene of ESBL-producing *E. coli* is the most prevalent plasmid antibiotic resistance gene. In this study, ESBL-producing *E. coli* was detected in 90% (54/60) of the chicken meat samples from Vietnam. Similar to our study, Le et al. (2015, 2016) found ESBL-producing *E. coli* isolates in 88.3% (Le et al. 2015), 58.7% (Le et al. 2015), and 92.7% (Nguyen et al. 2016) of Vietnamese chicken samples. The Vietnamese government is motivated towards researching antibiotic resistance and will be limiting the use of antibiotics in chicken farms. However, the prevalence of ESBL-producing *E. coli* has not changed, even after 5 years, and is considered to have become chronic due to its prevalence.

Previous studies by Le et al. (2015) and Nguyen et al. (2016) examined the CTX-M subgroup and found that *bla*_{CTX-M-1} group was the most common, followed by *bla*_{CTX-M-9} group. No actual identification was performed. In this study, the *bla*_{CTX-M-1} group was the most common, followed by *bla*_{CTX-M-9}. Among the *bla*_{CTX-M-1} group, *bla*_{CTX-M-55} was the most common, and among the *bla*_{CTX-M-9} group, *bla*_{CTX-M-65} was the most common. The results of a previous study of ESBL-producing *E. coli* from pork in Vietnam showed that *bla*_{CTX-M-55} was abundant, and the plasmid carrying *bla*_{CTX-M-55} was also found in workers and UTI patients (Hoang et al. 2017), suggesting plasmid transmission within the community. Based on these findings, *bla*_{CTX-M-55} is abundant in pork and chicken.

Multiple studies have reported on the genotyping of AmpC/ESBL-producing *E. coli* isolated from Japanese chickens. Kameyama et al. (2013) reported that the AmpC β -lactamase gene *bla*_{CMY-2} (66%) was the most prevalent, followed by *bla*_{CTX-M-1} (26%) and *bla*_{CTX-M-55} (26%). Hiroi et al. (2012) reported that ESBL-producing *E. coli* harbouring *bla*_{CTX-M-2} were most prevalent in chickens. Nahar et al. (2018) also reported that the *bla*_{CTX-M-2} group and the *bla*_{CTX-M-1} group respectively represented 45% and 34% of ESBL-producing *E. coli*. Our study found clear differences in prevalence of *bla*_{CTX-M} groups in Vietnamese and Japanese chicken samples. Hence, the pattern of *bla*_{CTX-M} varies between regions. In Vietnam, the co-harboring genes *bla*_{CTX-M-55} or *bla*_{CTX-M-65} with *bla*_{TEM} were most prevalent in ESBL-producing *E. coli*. These *bla*_{CTX-M} genes could be used to monitor antibiotic resistance genes towards improving healthcare in Vietnam.

The prevalence of antibiotic resistance genes is known to change annually (Bevan et al. 2017). The *bla*_{CTX-M} type of ESBL-producing *E. coli* isolated from humans is said to be *bla*_{CTX-M-15} (*bla*_{CTX-M-1} group) and *bla*_{CTX-M-14} (*bla*_{CTX-M-9} group), prevalent in many parts of the world. Accounting for prominent global trends in *bla*_{CTX-M} epidemiology, there is increasing evidence to suggest that *bla*_{CTX-M-27} has begun to outcompete other *bla*_{CTX-M} genotypes (Bevan et al. 2017). In Vietnam, a study of ESBL-producing *E. coli* isolates from healthy Vietnamese people from 2013–2015 reported that

*bla*_{CTX-M-27} was the most common, followed by *bla*_{CTX-M-55}, *bla*_{CTX-M-15}, and *bla*_{CTX-M-65} (Hoang et al. 2017). Our results show that in 2019, the overwhelming majority of *bla*_{CTX-M} isolates from chickens had ESBL-producing *E. coli* harbouring *bla*_{CTX-M-55} and *bla*_{CTX-M-65}. It has not been verified whether the *bla*_{CTX-M} gene is transferred from chickens to humans, but if it is, it is likely that *bla*_{CTX-M-55} or *bla*_{CTX-M-65} will be predominant in human carriers in the future. It is necessary to carefully monitor whether the detection of *bla*_{CTX-M-27} from ESBL-producing *E. coli* will increase in the future.

Declarations

Funding

This study was financially supported by the MEXT/JSPS KAKENHI Grant 18KK0168.

Conflict of Interest

None of the authors declares a conflict of interest.

Ethics approval and consent to participate

Ethics approval was not required for this research.

References

- Bevan ER, Jones AM, Hawkey PM (2017) Global epidemiology of CTX-M β -lactamases: temporal and geographical shifts in genotype. J Antimicrob Chemother 72:2145-2155. <http://doi: 10.1093/jac/dkx146>.
- Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ (2005) Identification of plasmids by PCR-based replicon typing. J Microbiol Methods 63:219-28.
- Chanawong A, M'Zail FH, Heritage J, Xiong JH, Hawkey PM (2002) Three cefotaximases, CTX-M-9, CTX-M-13, and CTX-M-14 among *Enterobacteriaceae* in the people's republic of China. Antimicrob. Agents Chemother 46:630-7. <http://doi: 10.1128/AAC.46.3.630-637.2002>.
- Giurazza R, Mazza MC, Andini R, Sansone P, Pace MC, Durante-Mangoni E (2021) Emerging Treatment Options for Multi-Drug-Resistant Bacterial Infections. Life (Basel) 11:519. <http://doi: 10.3390/life11060519>.
- Harada T, Yamane R, Dang VC, Nguyen DP, Nguyen TAD, Jinnai M, Yonogi S, Kawahara R, Kanki M, Kawai T, Kawatsu K, Kumeda Y, Isegawa Y, Yamamoto Y (2017) Prevalence and antimicrobial susceptibility of *Enterobacteriaceae* isolated from retail pepper in Vietnam. J Food Prot 80:716-24. <http://doi: 10.4315/0362-028X.JFP-16-501>.
- Hiroi M, Yamazaki F, Harada T, Takahashi N, Iida N, Noda Y, Yagi M, Nishio T, Kanda T, Kawamori F, Sugiyama K, Masuda T, Hara-Kudo Y, Ohashi N (2012) Prevalence of extended-spectrum beta-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae* in food-producing animals. J Vet Med Sci 74:189-95. <http://doi: 10.1292/jvms.11-0372>.
- Hoa TTT, Nakayama T, Huyen HM, Harada K, Hinenoya A, Phuong NT, Yamamoto Y (2020) Extended-spectrum beta-lactamase-producing *Escherichia coli* harbouring *sul* and *mcr-1* genes isolates from fish gut contents in the Mekong Delta, Vietnam. Lett Appl Microbiol 71:78-85. <http://doi: 10.1111/lam.13222>.
- Hoang TAV, Nguyen TNH, Ueda S, Le QP, Tran TTN, Nguyen TND, Dao TVK, Tran MT, Le TTT, Le TL, Nakayama T, Hirai I, Do TH, Vien QM, Yamamoto Y (2017) Common findings of *bla*_{CTX-M-55}-encoding 104-139 kbp plasmids harbored by extended-spectrum beta-lactamase-producing *Escherichia coli* in pork meat, wholesale market workers, and patients with urinary tract infection in Vietnam. Curr Microbiol 74:203-11. <http://doi: 10.1007/s00284-016-1174-x>.
- Imoto A, Ooi Y, Edogawa S, Ogura T, Masuda D, Mohamed M, Takii M, Umegaki E, Kawahara R, Ukimura A, Higuchi K (2014) Liver abscess caused by CTX-M-55type extended-spectrum beta-lactamase(ESBL)-producing *Salmonella enteritidis*. Intern Med 53:1699-703. <http://doi: 10.2169/internalmedicine.53.2407>.
- Kameyama M, Chuma T, Yabata J, Tominaga K, Iwata H, Okamoto K (2013) Prevalence and epidemiological relationship of CMY-2 AmpC beta-lactamase and CTX-M extended-spectrum beta-lactamase-producing *Escherichia coli* isolates from broiler farms in Japan. J Vet Med Sci 75:1009-15. <http://doi: 10.1292/jvms.12-0453>.
- Kawahara R, Fujiya Y, Yamaguchi T, Khong DT, Nguyen TN, Tran HT, Yamamoto Y (2019) Most domestic livestock possess colistin-resistant commensal *Escherichia coli* harboring *mcr* in a rural community in Vietnam. Antimicrob Agents Chemother 63:e00594-19. <http://doi: 10.1128/AAC.00594-19>.

- Le PQ, Awasthi SP, Hatanaka N, Hinenoya A, Hassan J, Ombarak RA, Iguchi A, Tran NTT, Dao KVT, Vien MQ, Le HX, Do HT, Yamamoto Y, Yamasaki S (2021) Prevalence of mobile colistin resistance (*mcr*) genes in extended-spectrum β -lactamase-producing *Escherichia coli* isolated from retail raw foods in Nha Trang, Vietnam. *Int J Food Microbiol* 346:109164. doi: 10.1016/j.ijfoodmicro.2021.109164.
- Liu YY, Wang Y, Walsh TR, Yi LX, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu LF, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu JH, Shen J (2016) Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 16:161-8. [http://doi: 10.1016/S1473-3099\(15\)00424-7](http://doi: 10.1016/S1473-3099(15)00424-7).
- Le QP, Ueda S, Nguyen TN, Dao TV, Van Hoang TA, Tran TT, Hirai I, Nakayama T, Kawahara R, Do TH, Vien QM, Yamamoto Y (2015) Characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* in retail meats and shrimp at a local market in Vietnam. *12:719-25*. <http://doi: 10.1089/fpd.2015.1954>.
- Le HV, Kawahara R, Khong DT, Tran HT, Nguyen TN, Pham KN, Jinnai M, Kumeda Y, Nakayama T, Ueda S, Yamamoto Y (2015) Widespread dissemination of extended-spectrum beta-lactamase-producing, multidrug-resistant *Escherichia coli* in livestock and fishery products in Vietnam. *J Food Contamin* 2:17. <http://DOI10.1186/s40550-015-0023-1>.
- Nahar A, Awasthi SP, Hatanaka N, Okuno K, Hoang PH, Hassan J, Hinenoya A, Yamasaki S. (2018) Prevalence and characteristics of extended-spectrum beta-lactamase-producing *Escherichia coli* in domestic and imported chicken meats in Japan. *J Vet Med Sci* 2018 80:510-517. <http://doi: 10.1292/jvms.17-0708>.
- Nakayama T, Jinnai M, Kawahara R, Diep KT, Thang NN, Hoa TT, Hanh LK, Khai PN, Sumimura Y, Yamamoto Y (2017) Frequent use of colistin-based drug treatment to eliminate extended-spectrum beta-lactamase-producing *Escherichia coli* in backyard chicken farms in Thai Binh Province, Vietnam. *Trop Anim Health Prod* 49:31-37. <http://doi: 10.1007/s11250-016-1154-y>.
- Nakayama T, Ueda S, Huong BTM, Tuyen LD, Komalamisra C, Kusolsuk T, Hirai I, Yamamoto Y (2015) Wide dissemination of extended-spectrum beta-lactamase-producing *Escherichia coli* in community residents in the Indochinese peninsula. *Infect and Drug Resist* 8:1-5. <http://doi: 10.2147/IDR.S74934>.
- Nguyen DP, Nguyen TAD, Le TH, Tran NMD, Ngo TP, Dang VC, Kawai T, Kanki M, Kawahara R, Jinnai M, Yonogi S, Hirai Y, Yamamoto Y, Kumeda Y (2016) Dissemination of extended-spectrum beta-lactamase-and AmpC beta-lactamase-producing *Escherichia coli* within the food distribution system of Ho Chi Minh City, Vietnam. *BioMed Research International* 2016:8182096. <http://doi: 10.1155/2016/8182096>.
- Nguyen TN, Khong DT, Le HV, Tran HT, Phan QN, Le HTT, Kawahara R, Yamamoto Y (2021) Quantitative Analysis of Colistin-Resistant *Escherichia coli* in Retail Meat from Local Vietnamese Markets. *Biomed Res Int* 19:2021:6678901. <http://doi: 10.1155/2021/6678901>.
- Noda T, Murakami K, Etoh Y, Okamoto F, Yatsuyanagi J, Sera N, Furuta M, Onozuka D, Oda T, Asai T, Fujimoto S (2015) Increase in resistance to extended-spectrum cephalosporins in *Salmonella* isolated from retail chicken products in Japan. *PLoS One* 10:e0116927. <http://doi: 10.1371/journal.pone.0116927>.
- Mathys DA, Mollenkopf DF, Feicht SM, Adams RJ, Albers AL, Stuever DM, Grooters SV, Ballash GA, Daniels JB, Wittum TE (2019) Carbapenemase-producing *Enterobacteriaceae* and *Aeromonas* spp. present in wastewater treatment plant effluent and nearby surface waters in the US. *PLoS One*. 14:e0218650. <http://doi: 10.1371/journal.pone.0218650>.
- Perez-Perez FJ, Hanson ND. (2002) Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. *J Clin Microbiol* 40:2153-62. <http://doi: 10.1128/JCM.40.6.2153-2162.2002>.
- Pitout JDD, Hossain A, Hanson ND (2004) Phenotypic and Molecular detection of CTX-M-beta-lactamases produced by *Escherichia coli* and *Klebsiella* spp. *J Clin Microbiol* 42:5715-21. <http://doi: 10.1128/JCM.42.12.5715-5721.2004>.
- Yamamoto Y, Kawahara R, Fujiya Y, Sasaki T, Hirai I, Khong DT, Nguyen TN, Nguyen BX (2019) Wide dissemination of colistin-resistant *Escherichia coli* with the mobile resistance gene *mcr* in healthy residents in Vietnam. *J Antimicrob Chemother* 74:523-524. doi: 10.1093/jac/dky435.
- Zhao S, Qaiyumi S, Friedman S, Singh R, Foley SI, White DG, McDermott PF, Donkar T, Bolin C, Munro S, Baron EJ, Walker RD (2003) Characterization of *Salmonella* enterica serotype newport isolated from humans and food animals. *J Clin Microbiol* 41:5366-71.
- Zhong H, Chen F, Li YJ, Zhao XY, Zhang ZL, Gu ZC, Yu YT (2021) Global trends and hotspots in research of carbapenem-resistant Enterobacteriaceae (CRE): a bibliometric analysis from 2010 to 2020. *Ann Palliat Med* 10:6079-6091. <http://doi: 10.21037/apm-21-87>.

Supplemental Table

Supplemental table1

		Primer	Sequence (5' to 3')	Reference
Phylogenetic group		ChuA. 1	GAC GAA CCA ACG GTC AGG AT	(Le HV. et al., 2015)
		ChuA. 2	TGC CGC CAG TAC CAA AGA CA	
		YjaA.1	TGA AGT GTC AGG AGA CGC TG	
		YjaA.2	ATG GAG AAT GCG TTC CTC AAC	
		TspE4C2.1	GAG TAA TGT CGG GGC ATT CA	
		TspE4C2.2	CGC GCC AAC AAA GTA TTA CG	
ESBL multiplex		TEM-410F	GGT CGC CGC ATA CAC TAT TCT C	(Le QP. et al., 2015)
		TEM-781R	TTT ATC CGC CTC CAT CCA GTC	
		SHV-287F	CCA GCA GGA TCT GGT GGA CTA C	
		SHV-517R	CCG GGA AGC GCC TCA T	
		ctxm1-115F	GAA TTA GAG CGG CAG TCG GG	
		ctxm1-702R	CAC AAC CCA GGA AGC AGG C	
		ctxm2-39F	GAT GGC GAC GCT ACC CC	
		ctxm2-145R	CAA GCC GAC CTC CCG AAC	
		ctxm9-16F	GTG CAA CGG ATG ATG TTC GC	
		ctxm9-490R	GAA ACG TCT CAT CGC CGA TC	
		ctxm8g25g-533F	GCG ACC CGC GCG ATA C	
		ctxm8g25g-718R	TGC CGG TTT TAT CCC CG	
<i>bla</i> _{CTX-M-1}	Detection	ctxm15-168F	TTC CAG AAT AAG GAA TCC CAT GG	(Imoto et al., 2014)
		ctx-m3-1059r	CCG TTT CCG CTA TTA CAA ACC G	
	Sequence	ctxm1-185F	TTT ATC GTG CTG ATG AGC GC	
		ctx-m3-492r	ACG GAA CGT TTC GTC TCC C	
<i>bla</i> _{CTX-M-2}	Detection	ctx-m2s-1f	ATG ATG ACT CAG AGC ATT	(In this study)
		ctx-m2-869f	CCG TGG GTT ACG ATT TTC GC	
	Sequence	toho1-1R	CGG TAG TAT TGC CCT TAA GCC	(Pitout et al., 2004)
		ctx-m2-316f	GTT AAC TAC AAT CCC ATT GCG GAG	(In this study)
<i>bla</i> _{CTX-M-9}	Detection	toho2-48F	CCGTATTGGGAGTTTGAGATGG	(Hoang et al., 2017)
		cxtm14-903R	AAAGTTCGATTTATTCAACAAAACCAG	
	Sequence	toho2-411F	GTCAACGGCACAATGACGC	
		ctxm9-490R	GAAACGTCTCATCGCCGATC	
AmpC multiplex		MOXMF	GCT GCT CAA GGA GCA CAG GAT	(Perez-Perez et al., 2002)
		MOXMR	CAC ATT GAC ATA GGT GTG GTG C	
		CITMF	TGG CCA GAA CTG ACA GGC AAA	
		CITMR	TTT CTC CTG AAC GTG GCT GGC	
		DHAMF	AAC TTT CAC AGG TGT GCT GGG T	
		DHAMR	CCG TAC GCA TAC TGG CTT TGC	
		ACCMF	AAC AGC CTC AGC AGC CGG TTA	
		ACCMR	TTC GCC GCA ATC ATC CCT AGC	
		EBCMF	TCG GTA AAG CCG ATG TTG CGG	
		EBCMR	CTT CCA CTG CGG CTG CCA GTT	

		FOXMF	AAC ATG GGG TAT CAG GGA GAT G	
		FOXMR	CAA AGC GCG TAA CCG GAT TGG	
<i>bla</i> _{CMY-2}	Detection	cmy-F	GAC AGC CTC TTT CTC CAC A	(Zhao et al., 2003)
		cmy-R	TGG AAC GAA GGC TAC GTA	
	Sequence	CMY2-outF	GTT ACA ATG TGT GAG AAG CAG TC	(Noda et al., 2015)
		CMY2-outR	ATG GGA TTT TCC TTG CTG TA	

Figures

Figure 1

Different CTX-M pattern of ESBL-producing *E. coli* isolates between Vietnam and Japan. (a) ESBL-producing *E. coli* isolates from chicken in Vietnam. (b) ESBL-producing *E. coli* isolates from chicken in Japan.

Figure 2

Antibiotic resistance pattern of *E. coli* isolates. (a) ESBL-producing *E. coli* isolates in Vietnam and (b) Japan. (c) *E. coli* harbouring *mcr-1* and (d) *E. coli* co-harbouring. Fisher's exact test was conducted. 1: significant different percentage of antibiotic resistance between *E. coli* isolated from Vietnam and Japan. 2: significant different percentage of antibiotic resistance between *E. coli* harbouring *mcr-1* with and without CTX-M. 3: significant different percentage of antibiotic resistance between ESBL-producing *E. coli* and *E. coli* harbouring *mcr-1* gene.

Figure 3

Multi-drug-resistance of *E. coli* harbouring *mcr-1* and Amp/ESBL-related genes