Infection and Drug Resistance

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ORIGINAL RESEARCH

Carriage of colistin-resistant, extended-spectrum β-lactamase-producing *Escherichia coli* harboring the *mcr-1* resistance gene after short-term international travel to Vietnam

This article was published in the following Dove Press journal:
Infection and Drug Resistance

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Background: Due to increasing colistin usage, the dissemination of the colistin-resistant gene *mcr-1* has been increasingly investigated. The aim of this study was to determine whether a traveler on a short-term international trip to a developing country could bring *mcr-1* back to their home country.

Materials and methods: Thirty-four travel events from Japan to Vietnam encompassing 19 travelers were assessed. A fecal specimen was collected from each traveler before and after each travel event and was inoculated on CHROMagar containing cefotaxime (CTX). Three to seven colonies exhibiting the characteristics of *Escherichia coli* were collected. Susceptibility to antibiotics and extended-spectrum β-lactamase (ESBL) production were determined by the disk diffusion method and the double-disk synergy test, respectively. ESBL-encoding genes were genotyped, and phylogenetic groupings were determined by multiplex polymerase chain reaction (PCR). The presence of *mcr-1* was also confirmed by PCR and sequencing.

Results: A total of 175 ESBL-producing *E. coli* isolated before and up to 2 weeks after traveling to Vietnam were analyzed. Genotyping of ESBL-producing isolates showed that $bla_{\text{CTX-M-1}}$/bla_{TEM} (27.7%) and $bla_{\text{CTX-M-9}}$ (45.9%) were the most prevalent genotypes, while the most frequently detected phylogenetic group was D (41.9%) followed by B2 (23.0%). In a significant number of travel events, travelers brought ESBL-producing *E. coli* back to Japan and three events by three travelers carried *mcr-1*. ESBL-producing *E. coli* isolates harboring *mcr-1* were identified as those carrying both $bla_{\text{CTX-M-14}}$ or $bla_{\text{CTX-M-55}}$ and *mcr-1*.

Conclusion: Using Vietnam as an example, we have shown that even a short-term trip to some countries may result in ESBL-producing *mcr-1*-positive *E. coli* carriage by international travelers.

Keywords: traveler, ESBL-producing *E. coli*, *mcr-1*, Vietnam, Japan

Introduction

With increasing globalization, people frequently travel to other countries. Under such circumstances, travelers can represent potential reservoirs for the dissemination of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae.[1]

Our previous study revealed that veterinarians in Vietnam prefer to prescribe colistin-based drugs for use on chicken farms.[2] Although colistin is effective for treating multidrug-resistant (MDR) bacteria, including carbapenem-resistant bacteria, this has resulted in the widespread dissemination of colistin-resistant bacteria harboring plasmids encoding the colistin-resistant gene, *mcr-1*.[3] Therefore, the prevalence of *mcr-1* requires investigation. Additionally, a recent report showed that colistin-resistant

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http://dx.doi.org/10.2147/IDR.S153178

Escherichia coli harboring *mcr-1* had been isolated from food animals as well as from farmers and urban persons in Vietnam.[4,5] Haenni et al[6] reported that the ESBL-related gene bla_{CTX-M} is encoded on mobile plasmids with *mcr-1*. Recently, transmission of colistin-resistant ESBL-producing *E. coli* by international travelers from China and India to developed countries has been reported.[7,8] Accordingly, we investigated whether travelers were bringing ESBL-producing *E. coli* harboring *mcr-1* back to their home countries after short-term stays in developing countries, such as Vietnam.

Materials and methods

A prospective cohort study was approved by the ethics committees of Osaka University (no eki31) and Osaka Prefectural Institute of Public Health (no 1602-02-2). All participants provided written informed consent. For any participant younger than 18 years, the parents provided written informed consent. A total of 34 travel events from Japan to Vietnam made by 19 travelers (age: 12–61 years, males: 11, length of stay in Vietnam: 2–12 days) between June 2015 and August 2016 were investigated.

An ~0.1 g fecal specimen was collected from each traveler within 6 days before and up to 21 days after each travel event, and 10 mL of sterile phosphate-buffered saline was added to each sample and homogenized. A 100 µL serially diluted homogenized solution was inoculated on CHROMagar™ ECC (Chromagar, Paris, France) containing 1 µg/mL of cefotaxime (CTX) and cultured at 37°C for 24 h. Three to seven colonies exhibiting the characteristics of *E. coli* were collected from each sample plate.

For *E. coli* identification, biochemical tests were conducted, including assays in lysine indole motility medium (Nissui, Tokyo, Japan), cellobiose lactose indole β-glucuronidase medium (Kyokuto Pharmaceutical Industrial, Tokyo, Japan), triple sugar iron slants (Nissui), and API 20E (bioMérieux, Marcy l'Etoile, France).

Susceptibility to antibiotics (ampicillin, fosfomycin, cefoxitin, CTX, ceftazidime, meropenem, gentamycin, kanamycin, streptomycin, tetracycline, ciprofloxacin, chloramphenicol, nalidixic acid, and sulfamethoxazole–trimethoprim) was determined by disk diffusion assay. ESBL production was determined by the double-disk synergy test using ceftazidime and CTX with and without clavulanic acid. Both antibiotic susceptibility and ESBL production tests were conducted as recommended by the Clinical and Laboratory Standards Institute.[9]

Bacterial DNA was extracted by boiling the bacterial suspension in tris(hydroxymethyl)aminomethane–ethylenediaminetetraacetic acid buffer. Genotyping of ESBL-encoding genes and phylogenetic groupings were determined by multiplex polymerase chain reaction (PCR).[10] The presence of the colistin-resistant gene *mcr-1* was also confirmed by PCR.[3] For both ESBL-encoding genes and *mcr-1*, the PCR products were identified by sequencing.[3,10] In *mcr-1*-positive strains, the colistin minimum inhibitory concentration (MIC) was evaluated by E-test® (bioMérieux).

The phylogenetic grouping of identified *E. coli* isolates was also determined by triplex PCR using a combination of two genes (*chuA* and *yjaA*) and the DNA fragment TSPE4.C2, as previously described.[11]

The clonal relationships of isolated *E. coli* strains were assessed by pulsed-field gel electrophoresis (PFGE), as previously described.[12]

The conjugation assay was performed as previously described.[13]

Statistical analysis was performed using Student's *t*-test. The significance level was set at $P<0.05$.

Results

A total of 207 CTX-resistant *E. coli* strains (43 and 164 before and after travel, respectively) were isolated from the fecal specimens of travelers before and up to 2 weeks after traveling to Vietnam. Among these isolates, 175 strains were identified as ESBL-producing *E. coli* (Table 1).

A significant number of travel events (88.2%, 30/34) by the 19 travelers brought ESBL-producing *E. coli* back to Japan, while in 32.3% (11/34) of travel events, it was detected before traveling in seven travelers (Table 1). Among these, 42.9% of ESBL-producing *E. coli* isolates showed the same PFGE pattern as before traveling.

Genotyping of ESBL-producing isolates showed that $bla_{CTX-M-1}$ group/bla_{TEM} and $bla_{CTX-M-9}$ group were the most prevalent genotypes, while $bla_{CTX-M-2}$ and $bla_{CTX-M-8}$ groups and bla_{SHV} were not detected in travelers. Phylogenetic analysis of ESBL-producing *E. coli* isolates was also conducted. The most frequently detected phylogenetic group was D (41.9%, 62/142) followed by B2 (23.0%, 34/142), while B1 (11.5%, 17/142) and A (16.2%, 24/142) were the least common.

ESBL-producing *E. coli* isolates showed 100% resistance to ampicillin and CTX. In the case of quinolones, ESBL-producing *E. coli* isolates exhibited a rate of resistance of 76.1% (108/142) to nalidixic acid, and >50% of isolates were resistant to sulfamethoxazole–trimethoprim (66.9%, 95/142) and tetracycline (52.1%, 74/142).

Moreover, three events by three travelers brought back *mcr-1*. The three travelers with *mcr-1*, who visited Vietnam

Table 1 Detection of ESBL-producing *Escherichia coli* harboring *mcr-1* before and after travel

Traveler	Traveling	Number of isolates			
		Before travel		After travel[a]	
		ESBL[b]	*mcr-1*[c]	ESBL	*mcr-1*
1	November 2015				
2	August 2016			3	
3	August 2016			3	
4	March 2016			6	
	May 2016	3		6	
5	May 2016			3	
6	May 2016			2	
7	February 2016				
8	August 2015	3		3	
	January 2016			1	
9	August 2015			7	
10	August 2015			7	I, B-1[d]
11	September 2015	2		6	
12	September 2015			7	
13	June 2015	4		7	
	September 2015	3		7	
	December 2015	3		3	
	January 2016	3		6	
14	July 2015	3			
	November 2015			5	
15	July 2015			6	
	November 2015			4	
16	June 2015			6	
	December 2015			3	
17	July 2015			3	
	September 2015			2	
	November 2015			5	
18	June 2015			6	
	July 2015	3		7	I, A-1
	September 2015	3		5	
	November 2015				
	January 2016			3	
19	September 2015			6	I, C-1
	January 2016	3		4	
Total			*		
19	34	33	0	142	3

Notes: [a]Specimens were collected up to 2 weeks after traveling. [b]Extended-spectrum β-lactamase-producing *E. coli*. [c]*mcr-1*-positive *E. coli*. [d]Strain name. *$P<0.001$.
Abbreviation: ESBL, extended-spectrum β-lactamase.

on business, did not carry *mcr-1* before traveling. ESBL-producing *E. coli* harboring *mcr-1* was identified by the co-occurrence of bla_{CTX-M} and *mcr-1*. Furthermore, the transmissibility of these *mcr-1* genes was confirmed by conjugation assay.

Characteristics of these three ESBL-producing *mcr-1*-positive *E. coli* isolates are summarized in Table 2. Traveler A, a 38-year-old man, traveled to Nha Trang by the way of Hanoi for 4 days. One day after returning to Japan, a fecal sample was collected and investigated for the presence of ESBL-producing *mcr-1*-positive *E. coli*, and three ESBL-producing isolates with different phylogenetic patterns were identified. These belonged to the $bla_{CTX-M-9}$ group and the phylogenetic group A, the $bla_{CTX-M-1}$ group/bla_{TEM} and the phylogenetic group D, and the $bla_{CTX-M-14}$ genotype and the phylogenetic group B2, the latter of which harbored *mcr-1*. However, no ESBL-producing *E. coli* harboring *mcr-1* was detected in a sample collected 17 days after returning to Japan. Traveler B traveled to Ho Chi Minh City for 12 days. Three days after returning to Japan, a fecal specimen was collected and four types of ESBL-producing *E. coli* were identified. These belonged to the $bla_{CTX-M-1}$ group and the phylogenetic group D, the $bla_{CTX-M-9}$ group and the phylogenetic group A, the $bla_{CTX-M-1}$ group/bla_{TEM} and the phylogenetic group D, and the $bla_{CTX-M-55}$/bla_{TEM} genotype and the phylogenetic group B2, the latter of which harbored *mcr-1*. Traveler C traveled to Can Tho by the way of Ho Chi Minh City for 5 days. Six days after returning to Japan, a fecal specimen was collected and four types of ESBL-producing *E. coli* were identified. These belonged to the $bla_{CTX-M-9}$ group and the phylogenetic group A, the $bla_{CTX-M-1}$ group and the phylogenetic group D, and the $bla_{CTX-M-14}$ genotype and the phylogenetic group D, the latter of which harbored *mcr-1*. In travelers B and C, no ESBL-producing *E. coli* harboring *mcr-1* was detected 20 and 19 days, respectively, after returning to Japan.

Discussion

Widespread dissemination of colistin-resistant MDR bacteria represents a worldwide threat owing to the limited number of effective antibiotics for treatment. In particular, the emergence of the mobile colistin-resistant gene *mcr-1* suggests the possibility of the rapid dissemination of MDR bacteria with colistin resistance in a community because of the potential for horizontal gene transfer. In these circumstances, international travelers could act as carriers, transferring *mcr-1*-positive MDR bacteria to their home countries. Even if only a limited number of travelers carry *mcr-1*-positive bacteria, the possibility of genetic transfer and expansion of *mcr-1* across borders seems to be likely. In this regard, there have been several reports of healthy travelers that carried ESBL-producing *E. coli* back to their home countries.[1,14] However, it is not clear whether a traveler on a short trip can carry *mcr-1* back to his/her home country.

In our previous reports, a high prevalence of human ESBL carriage (51%–71%) was demonstrated among Southeast Asian countries, such as Vietnam, Thailand, and Laos.[11] In contrast, the dissemination of ESBL-producing *E. coli* is limited in Japan, where there is a low prevalence of human ESBL carriers.[15] To date, only a limited number of bacterial isolates have been reported to harbor *mcr-1* in *E. coli* isolates from

Table 2 Characteristics of travelers carrying ESBL-producing *Escherichia coli* isolates harboring *mcr-1*

Variable	Traveler	A	B	C
Travelers	Age (years)	38	35	33
	Sex	Male	Male	Male
	Travel sites in Vietnam	Hanoi, Nha Trang	Ho Chi Minh City	Can Tho, Ho Chi Minh City
	Travel term	July 2015	August 2015	September 2015
	Length of stay in Vietnam	4 days	12 days	5 days
	Day of fecal sampling after returning to Japan	1 day	3 days	6 days
	Duration of carriage of *mcr-1* after returning to Japan	<17 days	<20 days	<19 days
Bacteria	Isolates	A-1	B-1	C-1
	Phylogenetic group	B2	B2	D
	Antibiotic resistance[a]	AMP, CTX, CHL, TET, GEN, KAN	AMP, CTX, SXT, TET	AMP, CTX, NAL, CHL, SXT, TET, STR
	ESBL CTX-M gene	$bla_{CTX\text{-}M\text{-}14}$	$bla_{CTX\text{-}M\text{-}55}$	$bla_{CTX\text{-}M\text{-}14}$
	Colistin resistance gene	*mcr-1*	*mcr-1*	*mcr-1*
	Colistin MIC (μg/mL)	1.0	3.0	4.0

Note: [a]Antibiotic resistance was determined by the Clinical & Laboratory Standards Institute guidelines: AMP, CTX, NAL, CHL, SXT, TET, GEN, KAN, and STR.
Abbreviations: AMP, ampicillin; CHL, chloramphenicol; CTX, cefotaxime; ESBL, extended-spectrum b-lactamase; GEN, gentamycin; KAN, kanamycin; MIC, minimum inhibitory concentration; NAL, nalidixic acid; STR, streptomycin; SXT, sulfa/trimethoprim; TET, tetracycline.

healthy animals, but in sick swine, there were high *mcr-1*-positive bacteria detected in Japan.[16,17]

It has been reported that in Hanoi, Vietnam, 37.5% of food animals are contaminated with *E. coli* harboring *mcr-1*.[4] In this study, we revealed that 15.8% of the travelers assessed became the carriers of ESBL-producing *E. coli* harboring *mcr-1* after a short stay in Vietnam. Even though the details of these carriage mechanisms are not clear, our results indicate that travelers may come to resemble Vietnamese residents, a percentage of whom are carriers of *mcr-1*-positive bacteria (unpublished data), during their stay in Vietnam. The three travelers who became *mcr-1* carriers ate breakfast in four-star hotels every morning, and they frequented middle-class restaurants for lunch and dinner. They experienced upper-class conditions during their stays in Vietnam and after returning to Japan. Their genotyping results were similar to those of healthy Vietnamese residents in terms of $bla_{CTX\text{-}M}$ genotypes, such as $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$ groups.[10] Conversely, an abundance of phylogenetic group D was observed in the isolates. However, the reason for this difference from healthy Vietnamese residents[10] was not clear. It may be due to differences in intestinal flora between Japanese travelers and Vietnamese residents.

More than 40% of isolates from each traveler before and after travel showed the same PFGE patterns, suggesting low genetic diversity within each traveler. Further study regarding the clonal relationships with residents may provide valuable information on the carriage of MDR bacteria by travelers.

Since the intestinal flora in travelers can be changed by diet and environment factors during travel, it may be responsible for the carriage of ESBL-producing *E. coli* with *mcr-1* after short-term travel.

Our follow-up study showed that the ESBL-producing *E. coli* harboring *mcr-1* disappeared in the three travelers, who were not treated with any antibiotics, within 3 weeks of returning home. Therefore, the stability of the carriage of *mcr-1*-positive bacteria may be limited if there is no exposure to antibiotics.

The three ESBL-producing *E. coli* isolates harboring *mcr-1* were further characterized, and the colistin MICs of the isolates were relatively low in comparison with those of isolates from food animals (4–8 μg/mL).[4] Since antibiotic MICs can be increased by treatment with a high concentration of another antibiotic, the acquisition of high colistin MICs may be possible.

Conclusion

The findings in this study suggest that even a short-term international trip to a developing country may result in ESBL-producing *mcr-1*-positive *E. coli* being carried back to the travelers' home countries. Although the dissemination of *E. coli* harboring *mcr-1* is limited in developing countries, the number of international travelers continues to increase, thereby increasing the risk of spreading *E. coli* harboring *mcr-1* into developed countries.

Acknowledgments

This work was supported by the Japan Agency for Medical Research and Development (AMED)/Japan International

Cooperation Agency (JICA) as a part of the Science and Technology Research Partnership for Sustainable Development (SATREPS), and MEXT/JSPS KAKENHI Grant 17H01687.

Disclosure

The authors report no conflicts of interest in this work.

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