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# Limited Transmission of *bla*$_{CTX-M-9}$-Type-Positive *Escherichia coli* between Humans and Poultry in Vietnam

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**We examined whether *Escherichia coli* isolates that produce CTX-M-9-type extended-spectrum β-lactamases (ESBL) are transferred between humans and chickens in a Vietnamese community. The phylogenetic group compositions, sequence types, antimicrobial resistance profiles, the prevalence of plasmid antibiotic resistance genes, and the plasmid replicon types generally differed between the human and chicken *E. coli* isolates. Our results suggest that transmission of the *bla*$_{CTX-M-9}$-positive *E. coli* between humans and poultry was limited.**

Extended-spectrum-β-lactamase (ESBL)-producing bacteria have been rapidly spreading worldwide over the last 30 years (1, 2). ESBL hydrolyzes third-generation cephalosporins and is produced in bacteria, such as *Escherichia coli* and *Klebsiella* spp. There are three main groups of ESBL genes: *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$. Recently, the CTX-M type of ESBL has become the most prevalent (1). So far, more than 100 genetic variants of *bla*$_{CTX-M}$ have been confirmed, and the genetic variants were categorized by their amino acids sequence into five main groups: CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25 types (2, 3).

The plasmids harboring most *bla*$_{CTX-M}$ and other antibiotics genes, such as *aac (3)-II*, *aac(6′)-Ib*, and *aph(3′)-I* (4), are transferable among bacterial cells. Therefore, it has been regarded as one of the major contributing factors to the rapid dissemination of multidrug-resistant bacteria.

Previous reports have indicated that the prevalence of ESBL-producing bacteria in healthy individuals is highest in China (50.5%) (5) and Thailand (65.7%) (6–8). Transmission mechanisms of these antibiotic-resistant bacteria are still unclear. Several previous reports suggest that companion animals might be reservoirs of the antibiotic-resistant bacteria in the community (9–12). However, direct transmission of antibiotic-resistant bacteria between humans and other animals has not been fully proved. Considering food-producing animals as one source of extraintestinal ESBL-producing bacteria to human has been a point of controversy (13). Therefore, in this study, we compared the antimicrobial susceptibility and the underlying genetic elements in the genomes of *bla*$_{CTX-M-9}$-positive *E. coli* isolated from stool samples provided by asymptomatic human individuals and obtained from poultry in a small Vietnamese village. We assessed whether the *bla*$_{CTX-M-9}$-positive *E. coli* was transferred between humans and poultry.

In this study, 199 fecal samples were collected from asymptomatic participants belonging to 47 households in a rural area of Vietnam (within a radius of approximately 150 m) from June 1 to 6 2013. At the same time, a rectal swab from a backyard chicken of each household was collected ($n$ = 47). Each fecal sample was directly inoculated on a MacConkey Agar plate containing 1 μg/ml cefotaxime. After an overnight incubation at 37°C, a typical single red colony of each sample was isolated and subjected to bacterial species determination and ESBL phenotype confirmation. The bacterial species were identified by the VITEK2 system (bioMérieux, Marcy l'Etoile, France). The ESBL phenotypes were confirmed by disc diffusion tests according to the Clinical and Laboratory Standards Institute standard M100-S23. Consequently, 93 (46.7%) and 20 (42.6%) *E. coli* isolates from 199 human and 47 chicken fecal samples, respectively, were confirmed as ESBL-phenotype-positive *E. coli* isolates. In this area, the CTX-M-9-type ESBL gene, *bla*$_{CTX-M-9}$ group, was most prevalent among CTX-M main groups (preliminary observation). Therefore, we confirmed CTX-M-9 group genes by PCR with TaKaRa EX *Taq* DNA polymerase (TaKaRa Bio Inc., Shiga, Japan) and primers targeting the *bla*$_{CTX-M-9}$ group (CTX-M-IV-SeqF [5′-TGTAACA CGGATTGACCGTAT-3′] and CTX-M-IV-SeqR-IS903 [5′-ACT CAGCAAAAGTTCGATTTATTC-3′]) according to the manufacturer's instruction. Consequently, the *bla*$_{CTX-M-9}$ type was detected in 64 (32.2%) and 15 (31.9%) of the 93 human and 20 chicken ESBL-phenotype-positive *E. coli* isolates, respectively. *E. coli* isolates other than the 64 and 15 *bla*$_{CTX-M-9}$-type-positive *E. coli* isolates possessed other *bla*$_{CTX-M}$ types than the *bla*$_{CTX-M-9}$ type (B. Ngan et al., unpublished data). In this study, the 93 human and 20 chicken *bla*$_{CTX-M-9}$-type-positive *E. coli* isolates were examined. The genes *aac (3)-II*, *aac(6′)-Ib*, *aadA*, *aph(3′)-I*, *cat1*, and *cmlA* were detected by PCR with heat-extracted bacterial DNA as the template (8, 14–16). Phylogenetic grouping of the *E. coli* isolates was determined by the protocol described by Clermont et al., using the three genetic markers *chuA*, *yjaA*, and TspE4.C2 (17). Plasmid replicons of plasmids harbored by the *E. coli* isolates were determined by following the original protocol

**Received** 3 March 2015 **Returned for modification** 5 March 2015
**Accepted** 9 March 2015

**Accepted manuscript posted online** 16 March 2015

**Citation** Ueda S, Ngan BTK, Huong BTM, Hirai I, Tuyen LD, Yamamoto Y. 2015. Limited transmission of *bla*$_{CTX-M-9}$-type-positive *Escherichia coli* between humans and poultry in Vietnam. Antimicrob Agents Chemother 59:3574–3577. doi:10.1128/AAC.00517-15.

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doi:10.1128/AAC.00517-15

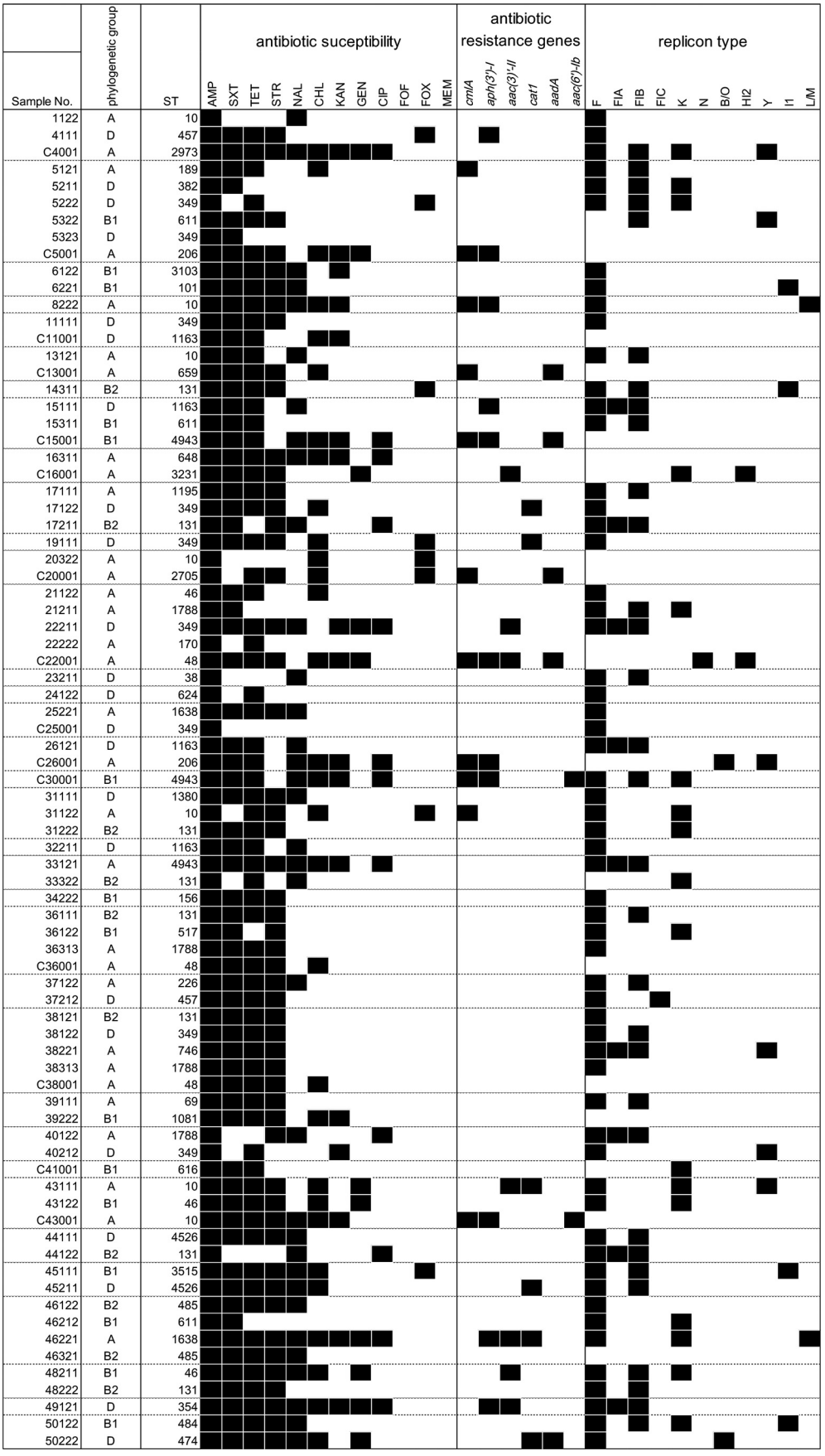


**FIG 1** Antibiotic susceptibility, antibiotic-related genes, and plasmid replicon type of the $bla_{\text{CTX-M-9}}$-positive *E. coli*. Black squares, resistant to antibiotic or detected each gene and replicon type.

**TABLE 1** Summary of MLST[a] analysis

| | *E. coli* isolates from: | | | | | |
|---|---|---|---|---|---|---|
| | All sources | | Human | | Chicken | |
| ST | No. | % | No. | % | No. | % |
| 349 | 9 | 11.4 | 8 | 12.5 | 1 | 6.7 |
| 131 | 8 | 10.1 | 8 | 12.5 | 0 | 0.0 |
| 10 | 7 | 8.9 | 6 | 9.4 | 1 | 6.7 |
| 1788 | 4 | 5.1 | 4 | 6.3 | 0 | 0.0 |
| 1163 | 3 | 3.8 | 2 | 3.1 | 1 | 6.7 |
| 46 | 3 | 3.8 | 3 | 4.7 | 0 | 0.0 |
| 611 | 3 | 3.8 | 3 | 4.7 | 0 | 0.0 |
| 48 | 3 | 3.8 | 0 | 0.0 | 3 | 20.0 |
| Other | 39 | 49.4 | 30 | 46.9 | 9 | 60.0 |
| Total | 79 | 100.0 | 64 | 100.0 | 15 | 100.0 |

[a] MLST, multilocus sequence typing.

described by Carattoli et al. (18). All isolates were typed by using a conventional multilocus sequence typing method described by Wirth et al. (19).

For nosocomial infections, clonal or oligoclonal distributions of antibiotic-resistant bacteria have often been observed (20). If the *E. coli* isolates obtained from the asymptomatic participants and their own domestic chickens were consistent with a clonal or oligoclonal distribution of a particular ancestral *E. coli* clone(s), similar phylogenetic relationships among the *E. coli* isolates would be observed for humans and chickens. The *E. coli* isolates from human were classified as groups A (32.8%), B1 (18.8%), B2 (15.6%), and D (32.8%). The isolates from chickens were primarily classified as group A (66.7%), and B1 (20.0%) and D (13.3%) were less frequent. Phylogenetic group B2, which has been regarded as including extraintestinal virulent strains, was not detected in the chicken isolates. Several sequence types (STs) were detected in the chicken and human isolates (Fig. 1). ST10 and ST349 were detected in the chickens and human isolates; however, antibiotic susceptibility, antibiotic resistance genes, and replicon type were not completely identical among the isolates with same STs (Table 1). There was no isolate set with the identical genomic characters of human and chicken isolates belonging to same household (Fig. 1).

We assessed the susceptibility of the $bla_{\text{CTX-M-9}}$-positive *E. coli* isolates by the standard disc diffusion test using Mueller-Hinton agar and 12 kinds of antibiotics discs (Eiken Chemical Co., Tokyo, Japan), including ampicillin (AMP), ciprofloxacin (CIP), chloramphenicol (CHL), fosfomycin (FOF), cefoxitin (FOX), gentamicin (GEN), kanamycin (KAN), meropenem (MEM), nalidixic acid (NAL), streptomycin (STR), trimethoprim-sulfamethoxazole (SXT), and tetracycline (TET) (Fig. 1). The proportions of *E. coli* isolates resistant to CHL and KAN were higher in chickens than in humans. None of the isolates was resistant to FOF or MEM. More than 80% of the isolates from humans and chickens were resistant to STR.

The genes *cmlA* and *cat1* confer resistance to CHL; *aac (3)-II* and *aac(6′)-Ib*, to GEN; *aph(3′)-I*, to KAN; and *aadA*, to STR (4). The prevalence of the *E. coli* isolates possessing at least one plasmid antibiotic resistance gene, such as *cmlA*, *cat1*, *aac (3)-II*, *aac(6′)-Ib*, *aph(3′)-I*, or *aadA*, was 21.9% in humans and 60.0% in chickens. The prevalence of each gene differed between human and chicken isolates. For example, 33.3% and 0.0% of the *E. coli* isolates from humans and chickens, respectively, possessed *cat1*. The CHL resistance gene *cmlA* was detected in both human and chicken isolates; however, *cat1* was detected only in human isolates (Fig. 1). In addition, the plasmid replicon types differed between the human and chicken *E. coli* isolates. Replicon type F was more prevalent in human isolates (87.5%) than in chicken isolates (20.0%). The replicons detected in chicken isolates included K, N, HI2, and Y. Furthermore, common combinations of replicon type and antibiotic resistance genes were not observed for human and chicken isolates.

Our results thus suggest that the transfer of the $bla_{\text{CTX-M-9}}$-type-positive *E. coli* isolate was very limited between humans and chickens in the rural Vietnamese community. However, our sample size was insufficient to exclude the possibility of transfer between human and poultry. Further studies with larger sample sizes will be important to address these issues.

## ACKNOWLEDGMENTS

This work was supported by the Japan Science and Technology Agency (JST)/Japan International Cooperation Agency (JICA) as part of the Science and Technology Research Partnership for Sustainable Development (SATREPS).

We thank the staff (N. T. A. Tuyet, H. T. T. Van, and P. T. T. Ha) at the Food Microbiology Department of the National Institute of Nutrition for their excellent technical assistance.

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