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Characteristics of CTX-M Extended-Spectrum β-Lactamase-Producing *Escherichia coli* Strains Isolated from Multiple Rivers in Southern Taiwan

Po-An Chen,[a,b] Chih-Hsin Hung,[c] Ping-Chih Huang,[d] Jung-Ren Chen,[e] I-Fei Huang,[a,b] Wan-Ling Chen,[a,b,f] Yee-Hsuan Chiou,[a,b,g] Wan-Yu Hung,[c] Jiun-Ling Wang,[h] Ming-Fang Cheng[a,b,g]

Department of Pediatrics, Kaohsiung Veterans General Hospital, Kaohsiung, Taiwan[a]; School of Medicine, National Yang-Ming University, Taipei, Taiwan[b]; Department of Chemical Engineering and Institute of Biotechnology and Chemical Engineering, I-Shou University, Kaohsiung, Taiwan[c]; Cheng-Shiu University, Kaohsiung, Taiwan[d]; Department of Biological Science and Technology, I-Shou University, Kaohsiung, Taiwan[e]; Department of Pediatrics, Pingtung Branch of Veterans General Hospital-Kaohsiung, Pingtung, Taiwan[f]; Fooyin University, Kaohsiung, Taiwan[g]; Department of Internal Medicine, National Cheng Kung University Hospital, Tainan, Taiwan[h]

Extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* sequence type ST131 has emerged as the leading cause of community-acquired urinary tract infections and bacteremia worldwide. Whether environmental water is a potential reservoir of these strains remains unclear. River water samples were collected from 40 stations in southern Taiwan from February to August 2014. PCR assay and multilocus sequence typing (MLST) analysis were conducted to determine the CTX-M group and sequence type, respectively. In addition, we identified the seasonal frequency of ESBL-producing *E. coli* strains and their geographical relationship with runoffs from livestock and poultry farms between February and August 2014. ESBL-producing *E. coli* accounted for 30% of the 621 *E. coli* strains isolated from river water in southern Taiwan. ESBL-producing *E. coli* ST131 was not detected among the isolates. The most commonly detected strain was *E. coli* CTX-M group 9. Among the 92 isolates selected for MLST analysis, the most common ESBL-producing clonal complexes were ST10 and ST58. The proportion of ESBL-producing *E. coli* was significantly higher in areas with a lower river pollution index ($P = 0.025$) and regions with a large number of chickens being raised ($P = 0.013$). ESBL-producing *E. coli* strains were commonly isolated from river waters in southern Taiwan. The most commonly isolated ESBL-producing clonal complexes were ST10 and ST58, which were geographically related to chicken farms. ESBL-producing *E. coli* ST131, the major clone causing community-acquired infections in Taiwan and worldwide, was not detected in river waters.

Escherichia coli sequence type ST131 (O25:H4), associated with CTX-M-15 extended-spectrum β-lactamase (ESBL), is the leading cause of community-acquired urinary tract infections (UTIs) and bacteremia worldwide (1–7). This clonal group is virulent and carries a broad range of resistance genes on transferable plasmids (1, 3–9). Studies conducted in southern Taiwan have reported that several clinical isolates of ESBL-producing *E. coli* belonged to the ST131-O25b lineage. The most prevalent ESBL-encoding gene is $bla_{\text{CTX-M-14}}$ (10, 11). Most patients with bacteremia or UTIs were healthy previously and did not exhibit any apparent risk factors. Only 30% of infants with UTIs caused by this clone had identifiable potential risk factors (e.g., previous antimicrobial use, hospitalization, neonatal infection, or underlying disease), suggesting that these UTIs were mostly community acquired, not hospital acquired (10–12).

The established reservoirs of the ST131 *E. coli* clone are humans, companion animals, noncompanion animals, and foods (7). Adequate studies have not been conducted for evaluating whether environmental water is a potential reservoir of this multidrug-resistant clone (13–15). Outbreaks of enteric infections are caused by various bacteria, protozoa, fungi, and viruses present in contaminated drinking water (16, 17). In addition, *E. coli* in irrigation water contaminates fresh produce (18; http://www.pma.com/~/media/pma-files/food-safety/cps/cps-research-reportag-water-200813version-11final.pdf). The contamination of rivers can occur through different sources, including human fecal contamination (sewer overflow during heavy rain or inadequate wastewater treatment), animals (particularly livestock), and runoff from pastures and sewage. In this study, we determined whether river water is a potential reservoir of ESBL-producing *E. coli* in southern Taiwan, focusing on the $bla_{\text{CTX-M}}$ clone found in human infections.

MATERIALS AND METHODS

Sampling of river water. The Taiwan Environmental Protection Administration (TEPA) routinely examines river water in southern Taiwan for the presence of heavy metals and *E. coli*. Figure 1 displays 99 stations located in the upper and lower streams in southern Taiwan that are monitored every month, including the Puzi, Bazhang, Jihsui, Tsengwen, Yanshui, Linbian, Donggang, Fenggang, Fangshan, Gaoping, Erren, Agongdian, and Shihchung Rivers and the Paoli and Gangkou streams. One hundred milliliters of river water was sampled each time and analyzed in accordance with the standard procedures of the Environmental Analysis

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Address correspondence to Ming-Fang Cheng, mfcheng@vghks.gov.tw, or Jiun-Ling Wang, dtmed111wang@ntu.edu.tw.

P.-A.C. and C.-H.H. contributed equally to this work.

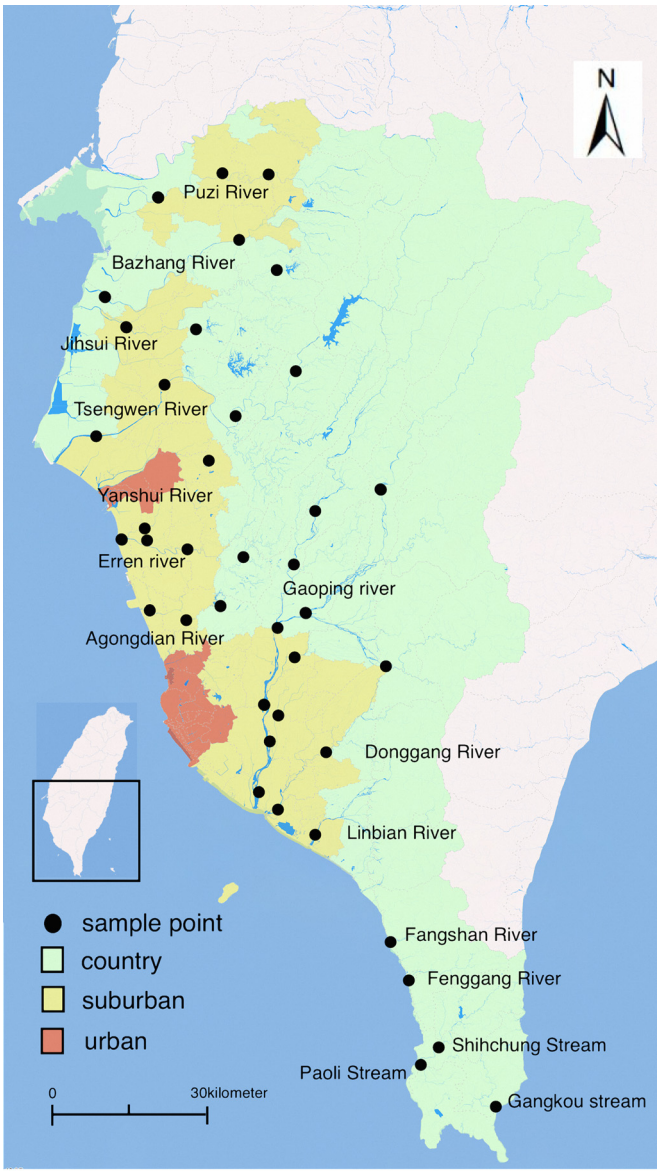

FIG 1 Study area in southern Taiwan, including the Puzi, Bazhang, Jihsui, Tsengwen, Yanshui, Linbian, Donggang, Fenggang, Fangshan, Gaoping, Erren, and Agongdian Rivers and the Shihchung, Paoli, and Gangkou streams, with 40 river sampling points. Map created via Paint 2, version 5.3.1 (250) (TryBest Studio), using data from the Taiwan National Land Surveying and Mapping Center.

Laboratory of the TEPA (19). All of the sampling procedures followed the rules established by the Environmental Analysis Laboratory of the TEPA. TPEA method NIEA W104.51C was used as the standard basis for sampling (19). Water samples were analyzed immediately after sample collection; the time required from sample collection to laboratory work completion was <24 h. To perform cluster sampling for each river and county, we used a random-number generator in selecting 40 of the 99 stations. One water sample was collected at each sampled station per month. In total, 280 river water samples were collected between February and August of 2014 in southern Taiwan. The normal mean daily temperature in the study area varied between 20.3°C in February and 28.7°C in August. The monthly precipitation ranged between 20.5 mm in February and 416.7 mm in August (http://www.cwb.gov.tw/V7/climate/monthlyMean/Taiwan_tx.htm).

Parameters of sample collection. Information on sampling sites, weather, human population grade, rainfall season, the river pollution index (RPI), distance to the river origin, and livestock and poultry densities around the sampling site were evaluated. In addition, the relationship between ESBL-producing *E. coli* isolates and these parameters was analyzed.

In accordance with the climate statistics provided by the Central Weather Bureau of Taiwan, the period from February to April was defined as the spring (dry) season, during which the mean temperature and precipitation of each month were <25°C and <100 mm, respectively. The period from May to August was defined as the summer (rainy) season, during which the mean temperature and precipitation of each month were >25°C and >100 mm, respectively. The human population around the sampling site and the population grade of each county or town were provided by the Ministry of the Interior of Taiwan. The population of a county or town was categorized as grade 1 for <49,000, grade 2 for between 49,000 and 93,000, grade 3 for between 93,000 and 137,000, and grade 4 for >137,000 people. The RPI was graded according to the definitions provided by the TEPA, which include the concentrations of 4 parameters in water: dissolved oxygen (DO), biochemical oxygen demand (BOD5), suspended solids (SS), and ammonia nitrogen (NH_3-N). We defined the RPI as the average of these 4 parameters, wherein an RPI of <2, 2 to 3, 3 to 6, and >6 was determined as nonpolluted, lightly polluted, moderately polluted, and severely polluted, respectively (20). We defined the upper, middle, and lower streams of a river based on the distance from the sampling site to the river origin, wherein the upper, middle, and lower streams were the first third, middle third, and final third of the river length, respectively. We collected information on livestock and poultry distribution from the Council of Agriculture, Taiwan. We defined totals of <100,000, between 100,000 and 500,000, and >500,000 chickens being raised per month as low, middle, and high densities of chicken farming, respectively. In a similar manner, we defined totals of <10,000, between 10,000 and 50,000, and >50,000 pigs being farmed per month as low, middle, and high densities of pig farming, respectively. We included 128 districts or counties in our study region. After excluding the area without chicken farms, we selected the tertiles as the cut points, which are approximately 100,000 and 500,000 chickens per month, for categorization. For pig farms, we selected 10,000 and 50,000 pigs per month as the cut points, because the chicken-farming density scale is approximately 10 times greater than the pig-farming density scale in southern Taiwan.

Identification and purification of *E. coli*. The river water samples were tested for *E. coli* by using the membrane filtration method. We used the decimal serial dilutions of river water filtered through 0.45-μm-pore-size membranes and followed the official method published by the TEPA (TEPA method NIEA E202.55B) (21). A series of 10-fold dilutions of the water sample were conducted using a sterile phosphate-buffered magnesium chloride solution as dilution blanks. The membranes were placed on an LES (Lawrence Experimental Station) endo agar plate (Bottle M Endo agar LES 500G; Becton Dickson and Company) and incubated at 35°C ± 1°C for 24 h. The *E. coli* was extracted from colonies that had a green metallic sheen and suspended in 1 ml of phosphate-buffered saline (PBS). We selected as many samples as possible and up to 10 colonies from each LES endo agar plate. One hundred microliters of the highest dilution displaying growth was spread on Luria-Bertani–ampicillin agar plates and incubated at 37°C overnight. Each of these colonies was subcultured 3 additional times. The purified isolates were inoculated on *E. coli* CHROMagar (ECC) plates (CHROMagar, Paris, France) (22) and further incubated overnight at 37°C to confirm the identification of *E. coli*.

CHROMagar ESBL plates (CHROMagar, Paris, France) were used to identify ESBL-producing *E. coli* according to the manufacturer's instructions.

Detection of O25b-ST131, multilocus sequence typing (MLST), and *bla*$_{CTX-M}$ gene groups. For chromosomal DNA preparation, a single colony of *E. coli* was suspended in 200 μl of sterile double-distilled water and

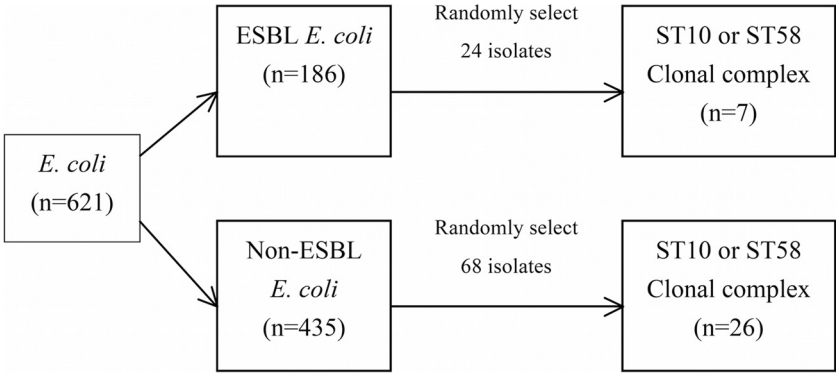

FIG 2 Sample selection for detecting the sequence type of *E. coli* from river waters in southern Taiwan.

heated to 100°C for 10 min. After cooling, the samples were centrifuged, and 2 μl of the supernatant containing chromosomal DNA was used as a template for PCR amplification.

The O25b serotype was determined by employing the methods of Clermont et al. (23), and the following screening primers were used: rfb.1bis (5′-ATACCGACGACGCCGATCTG-3′) and rfbO25b.r (5′-TGC TATTCATTATGCGCAGC-3′) (24, 25).

According to the stratification of the O25b status (Fig. 2), we used a random-number generator in selecting 92 isolates for MLST analysis. All isolates with the O25b serotype (12 ESBL-producing and 34 non-ESBL-producing) were evaluated through MLST analysis. Twelve O25-positive, ESBL-positive and 34 O25-positive, ESBL-negative *E. coli* strains were matched for randomly selecting O25-negative, ESBL-positive and O25-negative, ESBL-negative *E. coli* isolates.

The MLST grouping of *E. coli* was determined by analyzing 8 housekeeping gene sequences (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*) on the MLST database website (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli) (26). The eBURST (http://eburst.mlst.net/) algorithm was used to determine the clonal complexes of sequence types (STs) and sequence type complexes (STCs), where STs sharing at least 5 loci in common with at least one other member of the group were assigned to defined STCs; STs with the highest number of single-locus variants (SLVs) were assigned as the founder. In addition, the $bla_{\text{CTX-M}}$ gene of all ESBL-producing *E. coli* isolates was investigated. The CTX-M groups 1, 2, 8, and 9, as well as SHV and TEM, were detected by performing multiplex PCR with specific primers, as shown in Table 1 (26, 27). Specific PCRs were performed to detect $bla_{\text{CTX-M-14}}$ and $bla_{\text{CTX-M-15}}$ by employing the methods of Sidjabat et al. and Chia et al. (25, 26).

Statistical analysis. All statistical analyses were performed using the Statistical Package for Social Sciences (version 22.0) software package for Windows (SPSS Inc., Chicago, IL). Data concerning patients with ESBL-producing and non-ESBL-producing *E. coli* were expressed as percentages of each subgroup of patients and were compared by conducting the χ^2 test for categorical variables.

TABLE 1 O25b CTX-M group-specific primers used for multiplex PCR

PCR and primer names	Sequence[a] (5′–3′)	Reference
Multiplex II, CTX-M group 1, group 2, and group 9		
MultiCTXMGp1_for	TTAGGAARTGTGCCGCTGYA	27
MultiCTXMGp1-2_rev	CGATATCGTTGGTGGTRCCAT	
MultiCTXMGp2_for	CGTTAACGGCACGATGAC	
MultiCTXMGp1-2_rev	CGATATCGTTGGTGGTRCCAT	
MultiCTXMGp9_for	TCAAGCCTGCCGATCTGGT	
MultiCTXMGp9_rev	TGATTCTCGCCGCTGAAG	
CTX-M group 8/25		
CTX-Mg8/25_for	AACRCRCAGACGCTCTAC	
CTX-Mg8/25_rev	TCGAGCCGGAASGTGTYAT	
CTX-M-14		
CTX-14F	TACCGCAGATAATACGCAGGTG	26
CTX-14R	CAGCGTAGGTTCAGTGCGATCC	
CTX-M-15		
CTX-M-15-SF	CACACGTGGAATTTAGGGACT	25
CTX-M-15-SR	GCCGTCTAAGGCGATAAACA	
Multiplex I, TEM and SHV		
MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	27
MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC	
Multiplex PCR		
SHV-F	AACGGAACTGAATGAGGCGCT	26
SHV-R	TCCACCATCCACTGCAGCAGCT	

[a] Y = T or C; R = A or G; S = G or C; D = A, G, or T.

RESULTS

Identification of ESBL-producing *E. coli* in river water. ESBL-producing *E. coli* accounted for 186 (30%) of the 621 *E. coli* strains isolated from 280 river samples in southern Taiwan over the 7-month study period. All 621 *E. coli* isolates were screened for the O25b serotype through PCR assay. Serotype O25b accounted for 46 (7.4%) of the 621 isolates (Table 2). Although this serotype was observed more frequently among the ESBL-producing *E. coli* than among the non-ESBL-producing *E. coli* (12/186 [6.5%] versus 34/435 [2.8%]), the difference was statistically nonsignificant ($P = 0.55$).

As shown in Table 3, among the 12 ESBL-producing *E. coli* isolates belonging to the O25b serotype, 4 strains carried genes

TABLE 2 Numbers of O25b and non-O25b serotypes in ESBL-producing and non-ESBL-producing *E. coli*[a]

Serotype	No. of *E. coli* strains		Total
	ESBL ($n = 186$)	Non-ESBL ($n = 435$)	
O25b	12	34	46
Non-O25b	174	401	575
Total	186	435	621

[a] Numbers of O25b and non-O25b serotypes in ESBL-producing *E. coli* ($n = 186$) and non-ESBL-producing *E. coli* ($n = 435$) are from the 621 *E. coli* strains isolated from river waters in southern Taiwan.

TABLE 3 Number of CTX-M, SHV, and TEM genes detected in O25b ESBL-producing *E. coli* and non-O25b ESBL-producing *E. coli*[a]

Gene type	No. of genes in each *E. coli* serotype		
	O25b ESBL ($n = 12$)	Non-O25b ESBL ($n = 174$)	Total
CTX-M group 1	0	18	18
CTX-M group 2	2	1	3
CTX-M group 8	0	6	6
CTX-M group 9	1	42	43
SHV	0	23	23
TEM	7	89	96

[a] Shown are the numbers of genes of CTX-M group 1, group 2, group 8, and group 9 and SHV and TEM detected in O25b ESBL-producing *E. coli* ($n = 12$) and non-O25b ESBL-producing *E. coli* ($n = 174$) from 186 ESBL-producing *E. coli* strains isolated from river waters in southern Taiwan.

encoding CTX-M ESBLs, including 2 carrying the CTX-M group 2 gene, one carrying the CTX-M group 9 gene, and one carrying the CTX-M (3, 15) gene. Among the 186 ESBL-producing *E. coli* isolates, 18 carried the CTX-M group 1 gene, 3 carried the CTX-M group 2 gene, 6 carried the CTX-M group 8 gene, and 43 carried the CTX-M group 9 gene.

Among the 24 ESBL-producing *E. coli* isolates and 68 non-ESBL-producing *E. coli* isolates selected for MLST analysis, the sequence types were identified for 83 isolates. Genetic relatedness within each clonal complex is shown in Fig. 3 (based on data from the MLST database [http://mlst.warwick.ac.uk/mlst/]). Among the 83 *E. coli* isolates, the eBURST diagram revealed that the clustering of *E. coli* STs belonged to 6 main complexes (A, STC10; B, STC58; C, STC46; D, STC4623; E, STC641; and F, STC336); 15 isolates belonged to the ST10 clonal complex, and 18 belonged to the ST58 clonal complex (including 7 ST58 strains and 5 ST155 strains). No ST131 clonal complex was detected among all 92 *E. coli* isolates.

Among the identifiable 83 isolates detected from MLST analysis, 7 isolates belonged to the ST10 or ST18 clonal complex in the ESBL-producing group, and 26 isolates belonged to the ST10 or ST58 clonal complex in the non-ESBL-producing group.

Relationship between river water samples and evaluated parameters. The geographic distribution of the proportion of ESBL-producing *E. coli* in relation to the number of chickens being raised per month and RPI in southern Taiwan is displayed in Fig. 4. As shown in Table 4, no significant differences in the proportion of ESBL-producing *E. coli* strains were observed among the seasons, stream sites, populations, and numbers of pigs. The proportion of ESBL-producing *E. coli* was significantly higher in the low-RPI group ($P = 0.025$). In addition, the proportion of ESBL-producing *E. coli* strains was significantly higher in regions with a large number of chickens being raised ($P = 0.013$). No significant difference was observed in the proportion of the ST10 or ST58 clonal complex between the high- and low-density chicken farming areas ($P = 0.67$).

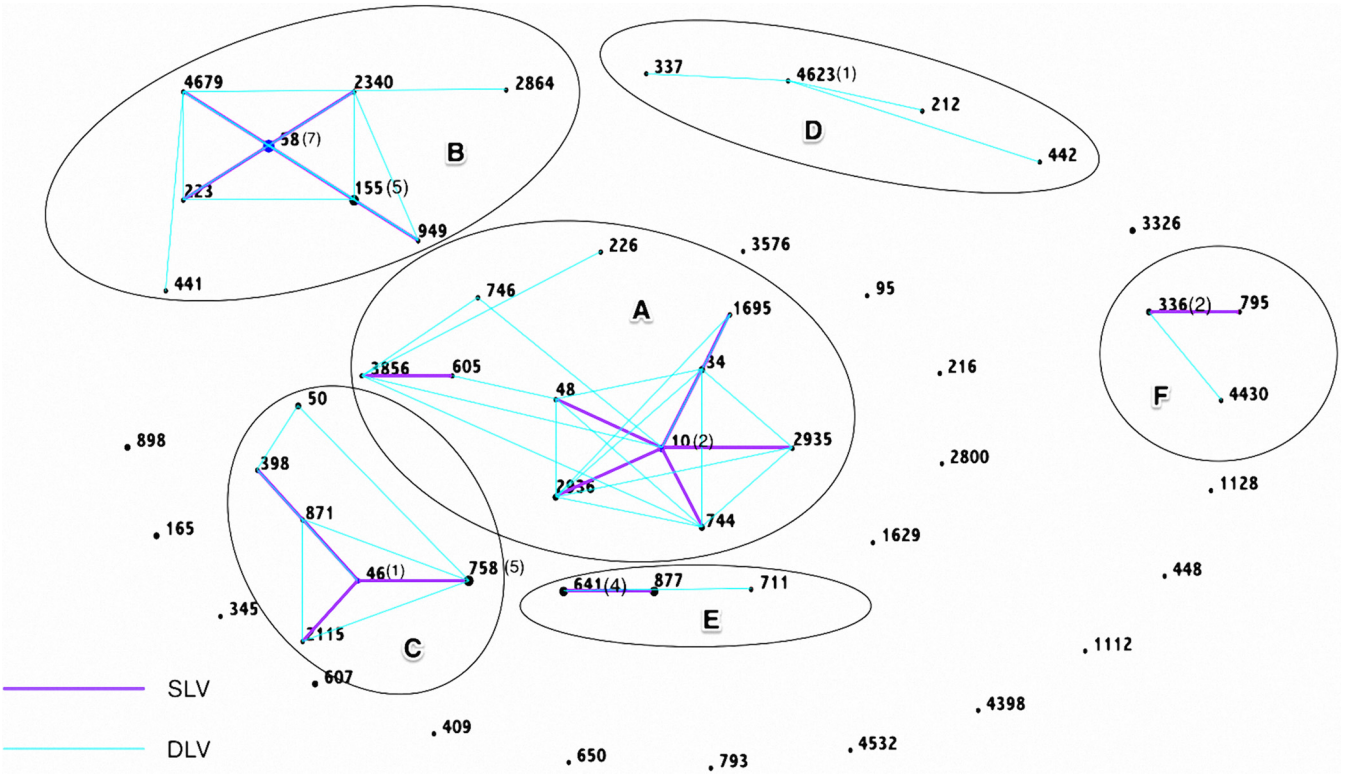

FIG 3 eBURST diagram showing the clustering of *E. coli* STs belonging to the 6 main complexes isolated from river waters in southern Taiwan: A, clonal complex of sequence type 10 (STC10; $n = 15$); B, STC58 ($n = 18$); C, STC46 ($n = 11$); D, STC4623 ($n = 4$); E, STC641 ($n = 9$); F, STC336 ($n = 4$). *E. coli* strains from the same ancestor based on the entire MLST data set were grouped and are indicated with black circles. Each ST is represented as a node. Single-locus variants (SLV) are connected with a purple line, and double-locus variants (DLV) are connected with a blue line. Strains that shared 5 of the 7 alleles were grouped into a single clonal complex.

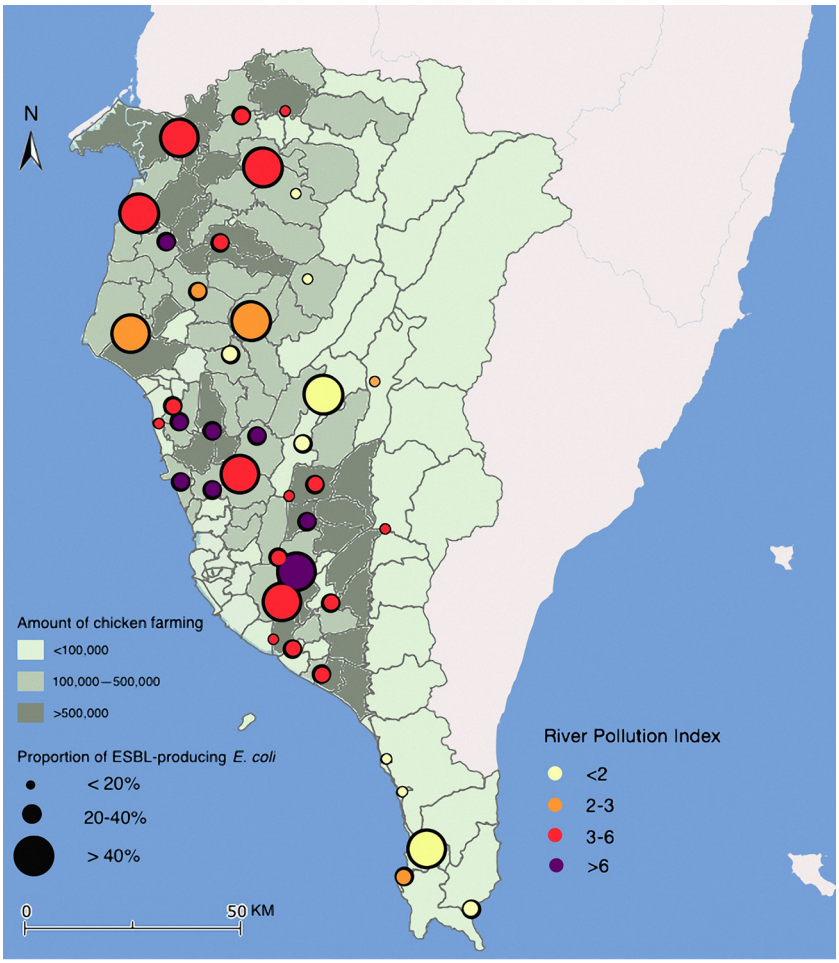

FIG 4 Geographic distribution of the proportion of ESBL-producing *E. coli* in relation to the number of chickens being raised and river pollution index (RPI) in southern Taiwan. The background of the map with the color gradient presents the number of chickens raised in each county; the dots with a size display the proportion of ESBL-producing *E. coli* in each sampling point, and the color of the dots represents the RPI. Map created via Paint 2, version 5.3.1 (250) (TryBest Studio), using data from the Taiwan National Land Surveying and Mapping Center.

DISCUSSION

Several studies have been conducted on the isolation of ESBL-producing *E. coli* from river water (13–15). The difference in the abundance of human and animal ESBL-producing *E. coli* clones in various river waters may be related to the nature of their drainage basins, the intensity of antibiotic use, and the relative amount of fecal contamination from humans and farm animals. Clonal groups similar to those found in human infections have been isolated from contaminated rivers adjacent to large cities (13–15). In contrast, nonhuman clonal groups more frequently are isolated from rural streams (28–30). The isolation rate of ESBL-producing *E. coli* was approximately 30% in our study, indicating their high numbers in environmental water. Zurfluh et al. reported an isolation rate of 36.2% for ESBL-producing *E. coli* from river and lake water in Switzerland (15). In a Chinese study, an isolation rate of 14.8% was reported for ESBL-producing *E. coli* from downstream water (31). However, research investigating river water as a potential reservoir of ESBL-producing *E. coli* is limited.

In our previous study, we observed that most ESBL-producing clinical *E. coli* strains isolated from infants belonged to CTX-M-14 in either ST131 or non-ST131 clones (10). In the current study, the most predominant clones observed were ST10 and ST58 clonal complexes. These ESBL-producing clones are associated with chickens and are unrelated to those found in human infections.

Colomer-Lluch et al. isolated quinolone-resistant *E. coli* belonging to the clonal groups O25b-H4 B2-ST131 and O25b:H4-D-ST69 from raw sewage and river water in Spain (13). In contrast, Dhanji et al. did not detect $bla_{\text{CTX-M-15}}$, the most common ESBL-encoding gene in clinical *E. coli* strains in the United Kingdom, in the Thames in London (14, 32). Geser et al. (33) identified CTX-M-1 ESBL-encoding genes in *Enterobacteriaceae* isolates from food-producing animals in Switzerland. However, the predominant ESBL-producing clinical isolates in Switzerland belonged to CTX-M-15 (34).

As shown in Table 5, various ESBL-producing *E. coli* clones have been isolated from animals, although ST131 clones have not been detected in certain studies (28, 30, 35–40, 42–45). The ST10, ST58, and ST155 clones have been detected in animals, water, and human isolates in the Netherlands, Canada, Chile, and China (28, 30, 35, 38–45). We detected numerous ST10 or ST58 clonal complexes in high-density chicken-farming areas. Additional studies

TABLE 4 Characteristics of ESBL-producing and non-ESBL-producing *E. coli* of 621 *E. coli* strains isolated from river waters in southern Taiwan

Characteristic	No. (%) of isolates		*P* value
	Non-ESBL ($n = 435$)	ESBL ($n = 186$)	
Season[a]			0.80
Dry (2–4 mo)	133 (70.7)	55 (29.3)	
Rain (5–8 mo)	302 (69.7)	131 (30.3)	
Region[b]			0.999
Upper	44 (69.8)	19 (30.2)	
Middle	166 (70.0)	71 (30.0)	
Lower	225 (70.1)	96 (29.9)	
Population[c]			0.19
≤137,000	423 (70.5)	177 (29.5)	
>137,000	12 (57.1)	9 (42.9)	
No. of animals being raised			
Chicken[d]			0.013
<100,000	131 (78.9)	35 (21.1)	
100,000–500,000	192 (66.2)	98 (33.8)	
>500,000	112 (67.9)	53 (32.1)	
Pig[e]			0.55
<1,000	205 (71.7)	81 (28.3)	
10,000–50,000	124 (67.0)	61 (33.0)	
>50,000	106 (70.7)	44 (29.3)	
RPI[f]			0.025
≤6	327 (67.8)	155 (32.2)	
>6	108 (77.7)	31 (22.3)	

[a] Dry season is defined from February to April and wet season from May to August.
[b] Water collected in the region is classified as upper, middle, and lower stream by the distance of the sampled station to the river origin.
[c] Classified by the population density at the sampled station.
[d] Classified by the number of chickens produced in the region of the sampled station in a month.
[e] Classified by the number of pigs produced in the region of sampled station in a month.
[f] Calculated by the average of the concentrations of four parameters in water: dissolved oxygen (DO), biochemical oxygen demand (BOD5), suspended solids (SS), and ammonia nitrogen (NH3-N).

are warranted to elucidate the virulence potential of *E. coli* ST10 and ST58 clonal groups.

We observed a significant association of ESBL-producing *E. coli* clones isolated from river water with the presence of chicken farms but not with pig farms ($P = 0.013$). This finding may be explained by the shorter period and higher antibiotic usage per average body weight for poultry compared to those for pigs. In southern Taiwan, antibiotic usage is 10 times higher in chickens than in pigs (42), although dosing is higher in pigs (43). According to the *Annual Report of Food and Drug Research of 2014*, released by the Food and Drug Administration, Taiwan, antibiotic residues were detected with greater frequency in chicken meat than in pig meat (46). In addition, a significant difference was observed in the proportion of ESBL-producing *E. coli* among different RPIs in our series ($P = 0.025$); this difference may be due to chicken farms being located mostly in suburban areas, where the river water is less polluted. The relationship between resistant bacteria and the RPI remains controversial. Multidrug-resistant bacteria in rivers may have developed because of the deterioration of water quality resulting from the flow of untreated sewage from domestic wastewater or intensive agricultural and industrial activities (47). However, the detection of multidrug-resistant bacteria in chlorinated water also has been reported (48, 49).

Our study has several limitations. Data from southern Taiwan may be inapplicable to other countries because of the geographic diversity of *E. coli* strains and their variable virulence and resistance genes. For example, ESBL-producing *E. coli* strains expressing $bla_{\text{CTX-M-14}}$ are prevalent in Taiwan, whereas those expressing $bla_{\text{CTX-M-15}}$ are more common in other parts of the world (11). Although we selected sample stations based on the surveillance data from the TEPA, water samples may not be independent because certain stations are located on the same river; however, the distribution of sampling stations is essential for the analysis of regional characteristics. Furthermore, the replication of *E. coli* in a single water sample is possible. We did not isolate the DNA of all *E. coli* strains, as has been conducted in previous studies; therefore, it is difficult to evaluate the replication (13, 15). Moreover, we did not detect ESBL-associated genes, including $bla_{\text{CTX-M}}$, bla_{SHV}, and bla_{TEM}, from certain ESBL-producing *E. coli* isolates in our study. We attribute this finding to the difference between the genes identified through PCR assay and the design of CHROMagar ESBL plates or other ESBL-associated genes, such as bla_{OXA}, which we did not detect. In addition, we did not detect several resistant genes reported in other studies; for instance, $bla_{\text{CMY-2}}$ may contribute to cefotaxime resistance. In an Asian surveillance study on ESBL-producing *Enterobacteriaceae*, isolates were reported to have a higher prevalence of AmpC bla_{CMY}-type β-lactamases, particularly $bla_{\text{CMY-2}}$, in Taiwan (50). Additional epidemiological studies are required to correlate river water, animal contact, poul-

TABLE 5 Summary of 13 major animal or human *E. coli* sequence type studies conducted after 2000

Author	Yr	Country	Sample origin (no.)	Most common serotype (%)	Dominant ST	ST131 (no.)	ST10 (no.)	ST58/ST155 (no.)	Reference
Overdevest et al.	2009	Netherlands	Chicken meat (89), beef (85), pork (57), mixed meat (22), other meat (9)	Chicken (CTX-M-1; 58.1), all other meat (CTX-M-1; 62.5)	ST117, ST10	Not found	Found	ST155	35
Ma et al.	2006	China	Duck feces (230), water (15)	CTX-M-27/55/24/105/14 (27.7/22/21.1/16.2/12.2)	NA	NA	NA	NA	36
Dierikx et al.	2009	Denmark	Broiler feces (1,066), farmer's feces (18)	CTX-M-1, CMY-2, SHV-12	ST93	Not found	Not found	Not found	37
Rashid et al.	2010	Bangladesh	OBS[a] feces (170), water (8)	CTX-M-15 (100)	ST156	Not found	ST10CC (1)	Not found	28
Hernandez et al.	2009	Chile	Gull feces (370), human feces (49)	CTX-M group 1 (94.6)	ST10	Not found	ST10 (24)	ST58 (4), ST155 (1)	30
Bergeron et al.	2005–2007	Canada	Human urine (475), meat of chicken (253), beef (242), pork (242), animal feces (349)	NA[b]	ST117, ST10	Not found	ST10(13)	Not found	38
Blaak et al.	2011	Netherlands	Flies (87), poultry manure in broiler farm (10), laying-hen farm (10)	SHV-12, CTX-M-1, TEM-52	ST58, ST10	Not found	ST10 (4)	ST58 (6), ST155 (2)	39
Cortes et al.	2003	Spain	Floor of poultry (59) and pig (27) farms	Poultry, CTX-M-9 group (64.9) and CTX-M-1 group (5.3); pig, CTX-M-1 group (69.0)	ST155, ST10	ST131 (1)	ST10 (3)	ST155 (4)	40
Peirano et al.	2000–2010	Canada	Human blood (4,698)	CTX-M-15, 119 (60); CTX-M-14, 58 (29)	ST131	ST131 (117)	ST10CC (13)	Not found	41
Leverstein-van Hall et al.	2006–2010	Netherlands	Chicken meat (98), floor of poultry farm (35), human (516)	Poultry, CTX-M-1 (49); chicken meat, CTX-M-1 (49); human, CTX-M-1 (24)	ST10, ST58	Not found	Human (3), meat (4)	Human, ST58 (3)	42
Hu et al.	2010–2013	China	Swine feces (31), water (26), human urine, blood, sputum, and body secretion (82)	CTX-M-1 group (33), CTX-M-9 (99)	ST131, CC10	ST131 (20)	CC10 (19)	NA	43
Ding et al.	2006–2007	China	Pig tissue (81)	NA	CC10	Not found	CC10 (26)	CC58 (8)	44
Borjesson et al.	2010	Sweden	Broiler feces (10)	CTX-M-1 (10)	ST155	Not found	Not found	ST155 (3)	45

[a] OBS, open bill stork (*Anastomus oscitans*).
[b] NA, not applicable.

try consumption, and other environmental exposures with the emergence of ESBL-producing *E. coli*.

In conclusion, this study investigated ESBL-producing *E. coli* in various river waters in southern Taiwan. We isolated several ESBL-producing *E. coli* clones found in poultry and other farm animals but not the O25b-ST131 clone, which causes human infections. These environmental ESBL-producing clones potentially can spread among humans, rendering treatment difficult. The continued surveillance of food and environment is essential to anticipate emerging infections.

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REFERENCES

1. **Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, Baquero F, Canton R, Nordmann P.** 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerg Infect Dis **14:**195–200. http://dx.doi.org/10.3201/eid1402.070350.
2. **Johnson JR, Menard M, Johnston B, Kuskowski MA, Nichol K, Zhanel GG.** 2009. Epidemic clonal groups of *Escherichia coli* as a cause of antimicrobial-resistant urinary tract infections in Canada, 2002 to 2004. Antimicrob Agents Chemother **53:**2733–2739. http://dx.doi.org/10.1128/AAC.00297-09.
3. **Lau SH, Kaufmann ME, Livermore DM, Woodford N, Willshaw GA, Cheasty T, Stamper K, Reddy S, Cheesbrough J, Bolton FJ, Fox AJ, Upton M.** 2008. UK epidemic *Escherichia coli* strains A-E, with CTX-M-15 beta-lactamase, all belong to the international O25:H4-ST131 clone. J Antimicrob Chemother **62:**1241–1244. http://dx.doi.org/10.1093/jac/dkn380.
4. **Nicolas-Chanoine MH, Blanco J, Leflon-Guibout V, Demarty R, Alonso MP, Canica MM, Park YJ, Lavigne JP, Pitout J, Johnson JR.** 2008. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. J Antimicrob Chemother **61:**273–281.
5. **Peirano G, Pitout JD.** 2010. Molecular epidemiology of *Escherichia coli* producing CTX-M beta-lactamases: the worldwide emergence of clone ST131 O25:H4. Int J Antimicrob Agents **35:**316–321. http://dx.doi.org/10.1016/j.ijantimicag.2009.11.003.
6. **Platell JL, Cobbold RN, Johnson JR, Heisig A, Heisig P, Clabots C, Kuskowski MA, Trott DJ.** 2011. Commonality among fluoroquinolone-resistant sequence type ST131 extraintestinal *Escherichia coli* isolates from humans and companion animals in Australia. Antimicrob Agents Chemother **55:**3782–3787. http://dx.doi.org/10.1128/AAC.00306-11.
7. **Rogers BA, Sidjabat HE, Paterson DL.** 2011. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother **66:**1–14. http://dx.doi.org/10.1093/jac/dkq415.
8. **Oteo J, Perez-Vazquez M, Campos J.** 2010. Extended-spectrum β-lactamase producing *Escherichia coli*: changing epidemiology and clinical impact. Curr Opin Infect Dis **23:**320–326. http://dx.doi.org/10.1097/QCO.0b013e3283398dc1.
9. **Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M.** 2010. *Escherichia coli* sequence type ST131 as the major cause of serious multidrug-resistant *E. coli* infections in the United States. Clin Infect Dis **51:**286–294. http://dx.doi.org/10.1086/653932.
10. **Cheng MF, Chen WL, Hung WY, Huang IF, Chiou YH, Chen YS, Lee SS, Hung CH, Wang JL.** 2015. Emergence of extended spectrum-beta-lactamase-producing *Escherichia coli* O25b-ST131: a major community-acquired uropathogen in infants. Pediatr Infect Dis J **34:**469–475.
11. **Chung HC, Lai CH, Lin JN, Huang CK, Liang SH, Chen WF, Shih YC, Lin HH, Wang JL.** 2012. Bacteremia caused by extended-spectrum-beta-lactamase-producing *Escherichia coli* sequence type ST131 and non-ST131 clones: comparison of demographic data, clinical features, and mortality. Antimicrob Agents Chemother **56:**618–622. http://dx.doi.org/10.1128/AAC.05753-11.
12. **Wu YH, Cheng MF, Lai CH, Lin HH, Hung CH, Wang JL.** 2014. The role of sequence type (ST) 131 in adult community-onset non-ESBL-producing *Escherichia coli* bacteraemia. BMC Infect Dis **14:**579. http://dx.doi.org/10.1186/s12879-014-0579-z.
13. **Colomer-Lluch M, Mora A, Lopez C, Mamani R, Dahbi G, Marzoa J, Herrera A, Viso S, Blanco JE, Blanco M, Alonso MP, Jofre J, Muniesa M, Blanco J.** 2013. Detection of quinolone-resistant *Escherichia coli* isolates belonging to clonal groups O25b:H4-B2-ST131 and O25b:H4-D-ST69 in raw sewage and river water in Barcelona, Spain. J Antimicrob Chemother **68:**758–765. http://dx.doi.org/10.1093/jac/dks477.
14. **Dhanji H, Murphy NM, Akhigbe C, Doumith M, Hope R, Livermore DM, Woodford N.** 2011. Isolation of fluoroquinolone-resistant O25b:H4-ST131 *Escherichia coli* with CTX-M-14 extended-spectrum beta-lactamase from UK river water. J Antimicrob Chemother **66:**512–516. http://dx.doi.org/10.1093/jac/dkq472.
15. **Zurfluh K, Hachler H, Nuesch-Inderbinen M, Stephan R.** 2013. Characteristics of extended-spectrum beta-lactamase- and carbapenemase-producing *Enterobacteriaceae* isolates from rivers and lakes in Switzerland. Appl Environ Microbiol **79:**3021–3026. http://dx.doi.org/10.1128/AEM.00054-13.
16. **Anonymous.** 2000. Waterborne outbreak of gastroenteritis associated with a contaminated municipal water supply, Walkerton, Ontario, May-June 2000. Canada Commun Dis Rep **26:**170–173.
17. **Hoxie NJ, Davis JP, Vergeront JM, Nashold RD, Blair KA.** 1997. Cryptosporidiosis-associated mortality following a massive waterborne outbreak in Milwaukee, Wisconsin. Am J Public Health **87:**2032–2035. http://dx.doi.org/10.2105/AJPH.87.12.2032.
18. **Steele M, Odumeru J.** 2004. Irrigation water as source of foodborne pathogens on fruit and vegetables. J Food Protect **67:**2839–2849.
19. **Taiwan Environmental Protection Administration.** 2004. Rivers, lakes, and reservoir water quality sampling general rule. TPEA method NIEA W104.51C. Taiwan Environmental Protection Administration, Taipei, Taiwan. (In Chinese.)
20. **Liou SM, Lo SL, Wang SH.** 2004. A generalized water quality index for Taiwan. Environ Monit Assess **96:**35–52. http://dx.doi.org/10.1023/B:EMAS.0000031715.83752.a1.
21. **Taiwan Environmental Protection Agency.** 2012. Water coliform detection method–filter method. TPEA method NIEA E202.55B. Taiwan Environmental Protection Agency, Taipei, Taiwan. (In Chinese.)
22. **Alonso JL, Soriano A, Carbajo O, Amoros I, Garelick H.** 1999. Comparison and recovery of *Escherichia coli* and thermotolerant coliforms in water with a chromogenic medium incubated at 41 and 44.5°C. Appl Environ Microbiol **65:**3746–3749.
23. **Clermont O, Dhanji H, Upton M, Gibreel T, Fox A, Boyd D, Mulvey MR, Nordmann P, Ruppe E, Sarthou JL, Frank T, Vimont S, Arlet G, Branger C, Woodford N, Denamur E.** 2009. Rapid detection of the O25b-ST131 clone of *Escherichia coli* encompassing the CTX-M-15-producing strains. J Antimicrob Chemother **64:**274–277. http://dx.doi.org/10.1093/jac/dkp194.
24. **Clermont O, Lavollay M, Vimont S, Deschamps C, Forestier C, Branger C, Denamur E, Arlet G.** 2008. The CTX-M-15-producing *Escherichia coli* diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J Antimicrob Chemother **61:**1024–1028. http://dx.doi.org/10.1093/jac/dkn084.
25. **Sidjabat HE, Paterson DL, Adams-Haduch JM, Ewan L, Pasculle AW, Muto CA, Tian GB, Doi Y.** 2009. Molecular epidemiology of CTX-M-producing *Escherichia coli* isolates at a tertiary medical center in western Pennsylvania. Antimicrob Agents Chemother **53:**4733–4739. http://dx.doi.org/10.1128/AAC.00533-09.
26. **Chia JH, Chu C, Su LH, Chiu CH, Kuo AJ, Sun CF, Wu TL.** 2005. Development of a multiplex PCR and SHV melting-curve mutation detection system for detection of some SHV and CTX-M beta-lactamases of *Escherichia coli*, *Klebsiella pneumoniae*, and *Enterobacter cloacae* in Taiwan. J Clin Microbiol **43:**4486–4491. http://dx.doi.org/10.1128/JCM.43.9.4486-4491.2005.

27. **Dallenne C, Da Costa A, Decre D, Favier C, Arlet G.** 2010. Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in Enterobacteriaceae. J Antimicrob Chemother **65:**490–495. http://dx.doi.org/10.1093/jac/dkp498.
28. **Rashid M, Rakib MM, Hasan B.** 2015. Antimicrobial-resistant and ESBL-producing *Escherichia coli* in different ecological niches in Bangladesh. Infect Ecol Epidemiol **5:**26712.
29. **Zhang H, Zhou Y, Guo S, Chang W.** 2015. Multidrug resistance found in extended-spectrum beta-lactamase-producing *Enterobacteriaceae* from rural water reservoirs in Guantao, China. Front Microbiol **6:**267.
30. **Hernandez J, Johansson A, Stedt J, Bengtsson S, Porczak A, Granholm S, Gonzalez-Acuna D, Olsen B, Bonnedahl J, Drobni M.** 2013. Characterization and comparison of extended-spectrum beta-lactamase (ESBL) resistance genotypes and population structure of *Escherichia coli* isolated from Franklin's gulls (*Leucophaeus pipixcan*) and humans in Chile. PLoS One **8:**e76150. http://dx.doi.org/10.1371/journal.pone.0076150.
31. **Gao L, Hu J, Zhang X, Ma R, Gao J, Li S, Zhao M, Miao Z, Chai T.** 2014. Dissemination of ESBL-producing *Escherichia coli* of chicken origin to the nearby river water. J Mol Microbiol Biotechnol **24:**279–285. http://dx.doi.org/10.1159/000365786.
32. **Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, Johnson AP, Pike R, Warner M, Cheasty T, Pearson A, Harry S, Leach JB, Loughrey A, Lowes JA, Warren RE, Livermore DM.** 2004. Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother **54:**735–743. http://dx.doi.org/10.1093/jac/dkh424.
33. **Geser N, Stephan R, Hachler H.** 2012. Occurrence and characteristics of extended-spectrum beta-lactamase (ESBL) producing *Enterobacteriaceae* in food producing animals, minced meat and raw milk. BMC Vet Res **8:**21. http://dx.doi.org/10.1186/1746-6148-8-21.
34. **Geser N, Stephan R, Korczak BM, Beutin L, Hachler H.** 2012. Molecular identification of extended-spectrum-beta-lactamase genes from *Enterobacteriaceae* isolated from healthy human carriers in Switzerland. Antimicrob Agents Chemother **56:**1609–1612. http://dx.doi.org/10.1128/AAC.05539-11.
35. **Overdevest I, Willemsen I, Rijnsburger M, Eustace A, Xu L, Hawkey P, Heck M, Savelkoul P, Vandenbroucke-Grauls C, van der Zwaluw K, Huijsdens X, Kluytmans J.** 2011. Extended-spectrum beta-lactamase genes of *Escherichia coli* in chicken meat and humans, The Netherlands. Emerg Infect Dis **17:**1216–1222. http://dx.doi.org/10.3201/eid1707.110209.
36. **Ma J, Liu JH, Lv L, Zong Z, Sun Y, Zheng H, Chen Z, Zeng ZL.** 2012. Characterization of extended-spectrum beta-lactamase genes found among *Escherichia coli* isolates from duck and environmental samples obtained on a duck farm. Appl Environ Microbiol **78:**3668–3673. http://dx.doi.org/10.1128/AEM.07507-11.
37. **Dierikx C, van der Goot J, Fabri T, van Essen-Zandbergen A, Smith H, Mevius D.** 2013. Extended-spectrum-beta-lactamase- and AmpC-beta-lactamase-producing *Escherichia coli* in Dutch broilers and broiler farmers. J Antimicrob Chemother **68:**60–67. http://dx.doi.org/10.1093/jac/dks349.
38. **Bergeron CR, Prussing C, Boerlin P, Daignault D, Dutil L, Reid-Smith RJ, Zhanel GG, Manges AR.** 2012. Chicken as reservoir for extraintestinal pathogenic *Escherichia coli* in humans, Canada. Emerg Infect Dis **18:**415–421. http://dx.doi.org/10.3201/eid1803.111099.
39. **Blaak H, Hamidjaja RA, van Hoek AH, de Heer L, de Roda Husman AM, Schets FM.** 2014. Detection of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* on flies at poultry farms. Appl Environ Microbiol **80:**239–246. http://dx.doi.org/10.1128/AEM.02616-13.
40. **Cortes P, Blanc V, Mora A, Dahbi G, Blanco JE, Blanco M, Lopez C, Andreu A, Navarro F, Alonso MP, Bou G, Blanco J, Llagostera M.** 2010. Isolation and characterization of potentially pathogenic antimicrobial-resistant *Escherichia coli* strains from chicken and pig farms in Spain. Appl Environ Microbiol **76:**2799–2805. http://dx.doi.org/10.1128/AEM.02421-09.
41. **Peirano G, van der Bij AK, Gregson DB, Pitout JD.** 2012. Molecular epidemiology over an 11-year period (2000 to 2010) of extended-spectrum beta-lactamase-producing *Escherichia coli* causing bacteremia in a centralized Canadian region. J Clin Microbiol **50:**294–299. http://dx.doi.org/10.1128/JCM.06025-11.
42. **Leverstein-van Hall MA, Dierikx CM, Cohen Stuart J, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJ, Mevius DJ, National ESBL Surveillance Group.** 2011. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. Clin Microbiol Infect **17:**873–880. http://dx.doi.org/10.1111/j.1469-0691.2011.03497.x.
43. **Hu YY, Cai JC, Zhou HW, Chi D, Zhang XF, Chen WL, Zhang R, Chen GX.** 2013. Molecular typing of CTX-M-producing *Escherichia coli* isolates from environmental water, swine feces, specimens from healthy humans, and human patients. Appl Environ Microbiol **79:**5988–5996. http://dx.doi.org/10.1128/AEM.01740-13.
44. **Ding Y, Tang X, Lu P, Wu B, Xu Z, Liu W, Zhang R, Bei W, Chen H, Tan C.** 2012. Clonal analysis and virulent traits of pathogenic extraintestinal *Escherichia coli* isolates from swine in China. BMC Vet Res **8:**140. http://dx.doi.org/10.1186/1746-6148-8-140.
45. **Borjesson S, Bengtsson B, Jernberg C, Englund S.** 2013. Spread of extended-spectrum beta-lactamase producing *Escherichia coli* isolates in Swedish broilers mediated by an incl plasmid carrying bla(CTX-M-1). Acta Vet Scand **55:**3. http://dx.doi.org/10.1186/1751-0147-55-3.
46. **Food and Drug Administration, Taiwan.** 2013. Annual report of drug residuals in animal product of 2014. Food and Drug Administration, Taipei, Taiwan. (In Chinese.)
47. **Al-Badaii F, Shuhaimi-Othman M.** 2015. Water pollution and its impact on the prevalence of antibiotic-resistant E. coli and total coliform bacteria: a study of the Semenyih River, peninsular Malaysia. Water Qual Exp Health **7:**319–330. http://dx.doi.org/10.1007/s12403-014-0151-5.
48. **Huang JJ, Hu HY, Tang F, Li Y, Lu SQ, Lu Y.** 2011. Inactivation and reactivation of antibiotic-resistant bacteria by chlorination in secondary effluents of a municipal wastewater treatment plant. Water Res **45:**2775–2781. http://dx.doi.org/10.1016/j.watres.2011.02.026.
49. **Murray GE, Tobin RS, Junkins B, Kushner DJ.** 1984. Effect of chlorination on antibiotic resistance profiles of sewage-related bacteria. Appl Environ Microbiol **48:**73–77.
50. **Sheng WH, Badal RE, Hsueh PR, SMART Program.** 2013. Distribution of extended-spectrum beta-lactamases, AmpC beta-lactamases, and carbapenemases among Enterobacteriaceae isolates causing intra-abdominal infections in the Asia-Pacific region: results of the Study for Monitoring Antimicrobial Resistance Trends (SMART). Antimicrob Agents Chemother **57:**2981–2988. http://dx.doi.org/10.1128/AAC.00971-12.