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Research Note

Characterization of Extended-Spectrum β-Lactamase–Producing *Escherichia coli* Isolates from Pigs and Farm Workers

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ABSTRACT

Food-producing animals can serve as reservoirs for extended-spectrum β-lactamase (ESBL)–producing *Escherichia coli*. The present study aimed to characterize and compare ESBL-carrying *E. coli* isolates from both pigs and farm workers. Rectal swabs were obtained from 60 pigs on four pig-fattening farms (15 samples per farm), and rectal swabs were taken from 40 farm workers on these farms (10 samples per farm). ESBL-carrying *E. coli* isolates from the workers and pigs were characterized by ESBL genotype, antibiotic susceptibility, enterobacterial repetitive intergenic consensus type, and multilocus sequence type. ESBL-producing *E. coli* was detected in 34 (56.7%) of 60 pigs, and 20.0% (8 of 40) of the farm workers were positive for ESBL-producing *E. coli*. More importantly, ESBL-producing *E. coli* isolates with the same β-lactamase genes, antibiotic resistance profiles, enterobacterial repetitive intergenic consensus types, and multilocus sequence types were detected in both pigs and workers on the same pig farm. These findings were suggestive for transfer of ESBL-producing *E. coli* between animals and humans.

Key words: *Escherichia coli*; Extended-spectrum β-lactamase; Farm workers; Pigs; Transfer

Extended-spectrum cephalosporins are frequently used to treat bacterial infections and are listed as important antimicrobials by the World Health Organization *(6)*. However, reports about extended-spectrum β-lactamase (ESBL)–producing bacteria isolated from both humans and animals have become increasingly frequent *(21)*. ESBLs are mainly plasmid-encoded enzymes that can hydrolyze the third- and fourth-generation cephalosporins and can be produced by a variety of bacteria, such as *Escherichia coli* and *Klebsiella pneumoniae,* the most commonly found ESBL-producing bacteria *(4, 10)*.

In recent years, ESBL-producing *E. coli* have frequently been isolated from food-producing animals across the world *(3, 7, 8, 11, 16, 22, 30)*. In China, an increasing number of ESBL-producing *E. coli* isolates have been recovered from food-producing animals since 2005 *(16, 22, 27, 30)*. Therefore, food animals have been regarded as a potential source for ESBL-producing *E. coli* and have attracted considerable attention *(23)*.

Of note, possible transmission of ESBL-producing *E. coli* from animal meat products to humans has been suggested *(13, 15, 20, 25, 26)*. ESBL-producing *E. coli* can also be transmitted from live animals to humans (or vice versa). Direct contact with live animals may be a potential risk factor for human ESBL carriage *(8)*. The present study was therefore designed to characterize and compare ESBL-producing *E. coli* isolates from both pigs and pig caretakers.

MATERIALS AND METHODS

Collection of rectal swab samples. During May to July 2015, on four pig-fattening farms (A, B, C, and D) in Shandong Province, People's Republic of China, rectal swab samples from pigs and farm workers were obtained and immediately transported into our laboratory for analysis within 6 h. Briefly, on each farm, rectal swabs from 15 pigs were randomly obtained by the farm veterinarians with sterile swabs, and rectal swab samples from 10 farm workers were collected by themselves using sterile swabs according to instructions by trained veterinarians. All participants gave written informed consent. The study protocol was approved by the Bio-medical Research Ethics Committee of Shandong Agricultural University (no. 2015-5-16).

Isolation and confirmation of ESBL-producing *E. coli*. All rectal swab samples were spread on MacConkey agar plates with added ceftriaxone (1 mg/liter) and were incubated at 37°C for 24 h. One presumptive colony with typical *E. coli* morphology was selected from each rectal swab sample and was identified using the API 20E system (bioMérieux, Marcy-l'Étoile, France). Suspected ESBL-producing *E. coli* isolates were confirmed by double-disk synergy test using both cefotaxime and ceftazidime in the presence or absence of clavulanic acid as recommended by the Clinical and Laboratory Standards Institute *(5)*.

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TABLE 1. *Primer pairs used for PCR amplification in this study*

Primer pair	Target gene or genetic element	Sequence (5′–3′)	Amplicon size (bp)	Annealing temp (°C)
CTX-M group 1 F	CTX-M group 1	TTA GGA ART GTG CCG CTG YA	415	57
CTX-M group 1 R		CGA TAT CGT TGG TGG TRC CAT		
CTX-M group 2 F	CTX-M group 2	CGT TAA CGG CAC GAT GAC	552	57
CTX-M group 2 R		CGA TAT CGT TGG TGG TRC CAT		
CTX-M group 9 F	CTX-M group 9	TCA AGC CTG CCG ATC TGG T	205	57
CTX-M group 9 R		TGA TTC TCG CCG CTG AAG		
TEM F	TEM	CAT TTC CGT GTC GCC CTT ATT C	840	56
TEM R		CGT TCA TCC ATA GTT GCC TGA C		
SHV F	SHV	AGC CGC TTG AGC AAA TTA AAC	1,051	56
SHV R		ATC CCG CAG ATA AAT CAC CAC		
ERIC F	ERIC	GTAAGCTCCTGGGGATTCAC		50
ERIC R		AAGTAAGTGACTGGGGTGAGCG		

Antimicrobial susceptibility testing. The disk diffusion method was used according to Clinical and Laboratory Standards Institute guidelines *(5)* to carry out antimicrobial susceptibility testing. A panel of 13 antimicrobial drugs was tested in this study, including ampicillin, cephalothin, cefotaxime, cefoxitin, imipenem, chloramphenicol, gentamicin, neomycin, streptomycin, tetracycline, ciprofloxacin, nalidixic acid, and trimethoprim-sulfamethoxazole. In addition, the MICs of four selected antimicrobials (cefotaxime, ceftazidime, cefepime, and imipenem), which are important for the scientific community to know, were tested using Etest strips (AB Biodisk), and the results were interpreted according to the Clinical and Laboratory Standards Institute guidelines *(5)*. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains.

Detection of β-lactamase gene. ESBL-producing *E. coli* isolates were analyzed for the presence of genes encoding TEM, SHV, and CTX-M β-lactamases by PCR as described previously (Table 1) *(1, 18, 30)*. The amplification conditions included an initial denaturation at 94°C for 5 min, 35 cycles of 94°C for 1 min, 56 or 57°C (depending on the primers) for 1 min, and 72°C for 1 min, with a final extension at 72°C for 7 min. The PCR products were sequenced using an ABI 3730 automated sequencer (BaseClear, Leiden, The Netherlands). Forward and reverse sequences were aligned with reference sequences in the BLAST database (http://blast.ncbi.nlm.nih.gov/Blast.cgi) to confirm the β-lactamase type (http://www.ncbi.nlm.nih.gov/projects/pathogens/submit_beta_lactamase).

ERIC PCR. The genetic similarity of ESBL-producing *E. coli* isolates from both pigs and farm workers was analyzed using enterobacterial repetitive intergenic consensus (ERIC)–based primers for PCR, as previously described (Table 1) *(17, 19)*. The PCR protocol included an initial denaturation (3 min at 94°C), 30 denaturation cycles (1 min at 94°C), annealing (1 min at 50°C), chain extension (3 min at 72°C), and a final extension (5 min at 72°C). The PCR products were separated in 2% agarose gels by electrophoresis. NTSYSpc software (version 2.10) was used to produce a dendrogram, and clustering analysis was performed by the unweighted pair group method with arithmetic averages according to the Dice similarity index *(17)*.

MLST. Forty-two ESBL-producing *E. coli* isolates recovered from both pigs and farm workers were subjected to multilocus sequence type (MLST) analysis *(8)*. Briefly, MLST was carried out using seven conserved housekeeping genes (*adk, fumC, gyrB, icd, mdh, purA,* and *recA*) (http://mlst.warwick.ac.uk/mlst/).

RESULTS

ESBL-producing *E. coli* isolates from pigs and farm workers. A total of 42 ESBL-producing *E. coli* isolates were obtained in this study. Thirty-four ESBL-producing *E. coli* isolates (34 of 60, 56.7%) were obtained from four pig farms, including 10 isolates from farm A (10 of 15, 66.7%), 10 from farm B (10 of 15, 66.7%), 9 from farm C (9 of 15, 60.0%), and 5 from farm D (5 of 15, 33.3%). Eight ESBL-producing *E. coli* isolates were isolated from 40 farm workers (8 of 40, 20.0%), including 3 workers from farm A (3 of 10, 30.0%), 2 from B (2 of 10, 20.0%), 2 from C (2 of 10, 20.0%), and 1 from D (1 of 10, 10.0%) (Fig. 1).

Antimicrobial susceptibility. All 42 ESBL-producing *E. coli* isolates from both pigs and farm workers were resistant to ampicillin, cephalothin, and cefotaxime. Fortunately, these isolates were all susceptible to imipenem and cefoxitin. Among the 34 ESBL-producing *E. coli* isolates from pigs, most of the isolates showed resistance to gentamicin (30 of 34, 88.2%), tetracycline (30 of 34, 88.2%), streptomycin (24 of 34, 70.6%), and trimethoprim-sulfamethoxazole (24 of 34, 70.6%); moderate resistance rates were observed for nalidixic acid (20 of 34, 58.8%), chloramphenicol (18 of 34, 52.9%), and neomycin (14 of 34, 41.2%); and relatively low resistance rates were observed for ciprofloxacin (7 of 34, 20.6%). Among the eight ESBL-producing *E. coli* isolates from farm workers, high resistance rates were observed for tetracycline (6 of 8, 75.0%) and nalidixic acid (6 of 8, 75.0%); moderate resistance rates were observed for gentamicin (4 of 8, 50.0%), neomycin (4 of 8, 50.0%), streptomycin (4 of 8, 50.0%), and trimethoprim-sulfamethoxazole (4 of 8, 50.0%); and relatively low resistance rates were observed for chloramphenicol (3 of 8, 37.5%) and ciprofloxacin (3 of 8, 37.5%) (Fig. 1). The MIC ranges for cefotaxime, ceftazidime, cefepime, and imipenem were 4 to $\geq$16 μg/ml, $\leq$0.5 to 32 μg/ml, 0.5 to 16 μg/ml, and 0.19 to 0.25 μg/ml, respectively.

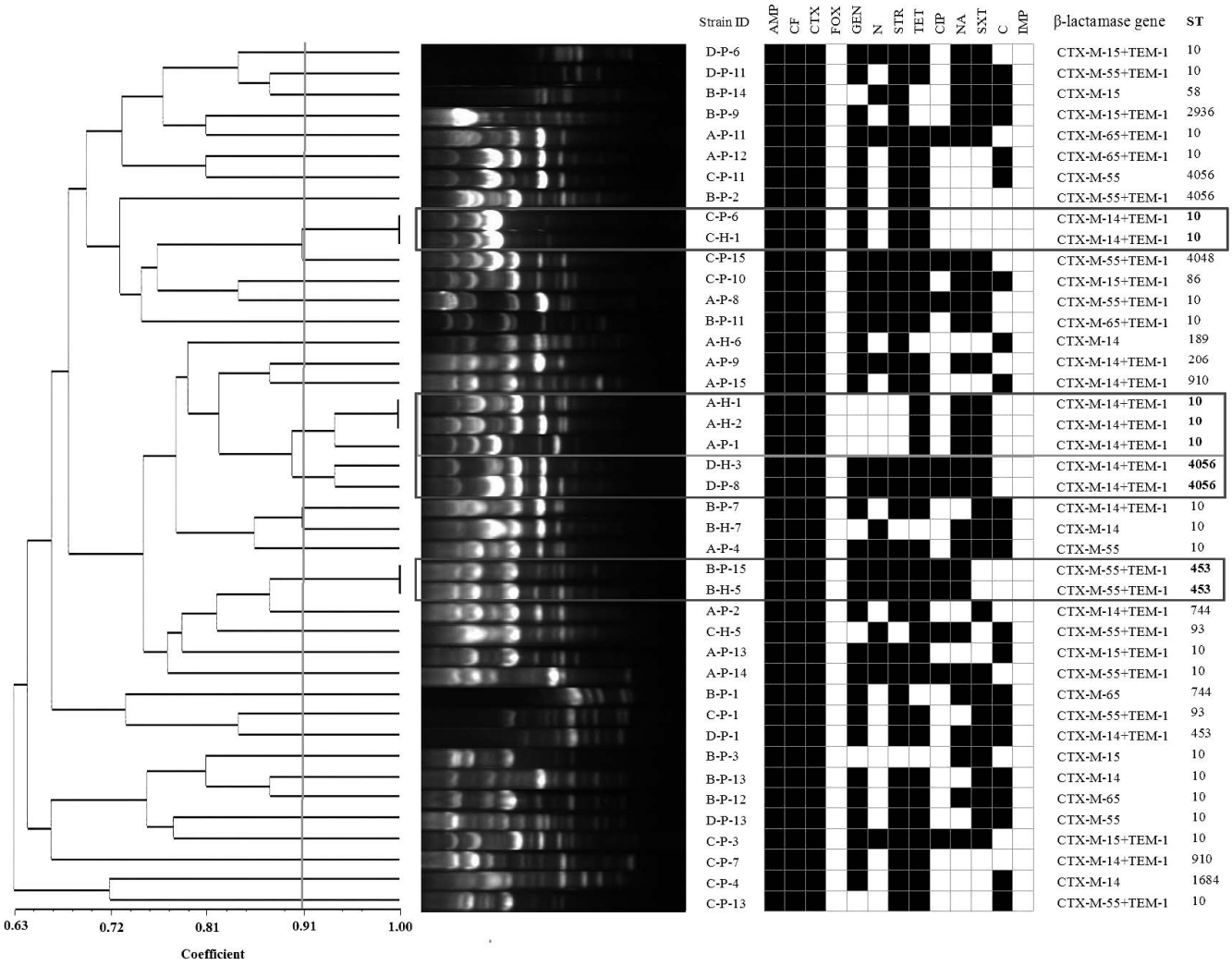

FIGURE 1. *Characteristics of ESBL-producing* E. coli *isolated in this study, including origins, antimicrobial resistances, β-lactamase genes, MLST, and ERIC-PCR fingerprints. A-, B-, C-, and D-P, ESBL-producing* E. coli *isolates from pigs on farms A to D; A-, B-, C-, and D-H, ESBL-producing* E. coli *isolates from farm workers on farms A to D; black, resistant; white, susceptible; AMP, ampicillin; CF, cephalothin; CTX, cefotaxime; FOX, cefoxitin; GEN, gentamicin; N, neomycin; STR, streptomycin; TET, tetracycline; CIP, ciprofloxacin; NA, nalidixic acid; SXT, trimethoprim-sulfamethoxazole; C, chloramphenicol; IMP, imipenem.*

Characterization of β-lactamase genes. All 42 ESBL-producing *E. coli* isolates from both pigs and workers carried $bla_{\text{CTX-M}}$ genes. However, no bla_{SHV} genes were detected in this study. Among the 34 ESBL-producing *E. coli* isolates from pigs, the dominant $bla_{\text{CTX-M}}$ type was $bla_{\text{CTX-M-55}}$ (11 of 34, 32.4%), followed by $bla_{\text{CTX-M-14}}$ (10 of 34, 29.4%), $bla_{\text{CTX-M-15}}$ (7 of 34, 20.6%), and $bla_{\text{CTX-M-65}}$ (5 of 34, 14.7%). Of note, 25 ESBL-producing *E. coli* isolates (73.5%) from pigs contained both $bla_{\text{CTX-M}}$ and $bla_{\text{TEM-1}}$. Among the eight ESBL-producing *E. coli* isolates from farm workers, the dominant $bla_{\text{CTX-M}}$ type was $bla_{\text{CTX-M-14}}$ (6 of 8, 75.0%), followed by $bla_{\text{CTX-M-55}}$ (2 of 8, 25.0%). Additionally, six of the eight ESBL-producing *E. coli* isolates (75.0%) from farm workers contained both $bla_{\text{CTX-M}}$ and $bla_{\text{TEM-1}}$ (Fig. 1).

ERIC and MLST. Thirteen different MLSTs were detected in isolates from both farm workers and pigs. Twenty-two of 42 ESBL-producing *E. coli* isolates belonged to sequence type 10 (ST-10). More importantly, four sets of ESBL-producing *E. coli* isolates from both farm workers and pigs with the same β-lactamase genes and antibiotic resistance profiles were identical in their MLSTs and ERIC types. Specifically, at pig farm A, two farm worker isolates and one pig isolate were identified as *E. coli* ST-10; at pig farm B, one farm worker isolate and one pig isolate were identified as *E. coli* ST-453; at pig farm C, one farm worker isolate and one pig isolate were identified as *E. coli* ST-10; and at pig farm D, one farm worker isolate and one pig isolate were identified as *E. coli* ST-4056 (Fig. 1).

DISCUSSION

In this study, ESBL-producing *E. coli* isolates with the same β-lactamase genes, antibiotic resistance profiles, ERIC types, and MLSTs were detected in pigs and farm workers on the same farms, indicating possible transfer of ESBL-producing *E. coli* between pigs and farm workers. Results from other studies are also suggestive for transmission of

ESBL-producing *E. coli* between animals and humans *(8, 11)*. Additionally, the ERIC PCR results revealed genetic heterogeneity of ESBL-producing *E. coli* isolates among the different pig farms. However, a few clusters with high similarity (≥90%) composed of isolates from different pig farms were also detected. The isolates within these clusters were subjected to MLST analysis, which confirmed strain relatedness in some but not all cases. Therefore, ERIC PCR in combination with MLST can be considered as an appropriate molecular method to analyze genetic relatedness among ESBL-producing *E. coli* isolates.

All 42 isolates from both pigs and farm workers in our study were resistant to three or more of the antibiotics tested, which defines them as multidrug resistant. Of note, the 42 isolates were all resistant to ampicillin, cephalothin, and cefotaxime. This result may be mainly associated with the fact that ampicillin, cephalothin, and cefotaxime have been frequently used in local human and animal clinical practices. Fortunately, these isolates were all susceptible to imipenem and cefoxitin.

Food-producing animals have been recognized as important carriers of ESBL-producing *E. coli (2)*. A connection between ESBL-producing *E. coli* in food production animals, chicken meat, and humans has been suggested *(14),* and several studies revealed the presence of community-associated ESBL-producing *E. coli* infection among healthy humans *(9, 28)*. Thus, the high rate of isolation of ESBL-producing *E. coli* from pigs (56.7%) found in this study is of great concern. Of note, extended-spectrum cephalosporins, such as cephalothin and cefotaxime, have frequently been used on these pig farms, which may contribute to the high rate of isolation of ESBL-producing *E. coli*.

All 34 ESBL-producing *E. coli* isolates from pigs harbored *bla* genes, and $bla_{\text{CTX-M}}$ was the dominant ESBL gene, which is similar to findings reported previously in China *(22, 29)*. Among the 34 ESBL-producing *E. coli* isolates recovered from pigs, a high diversity of CTX-M variants was observed. $bla_{\text{CTX-M-55}}$ (32.4%) and $bla_{\text{CTX-M-14}}$ (29.4%) were the most common genes detected that confer resistance to cephalosporins, in agreement with several reports from China *(22, 29)*. Additionally, $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-65}}$ were detected in ESBL-producing *E. coli* isolates from pigs.

In the current study, the isolation rate for ESBL-producing *E. coli* in farm workers was 20.0%, higher than the reported isolation rate of 7.0% in rural inhabitants in Shandong Province, China *(28)*. This result suggested that frequent contact with animals carrying ESBL-producing *E. coli* may be a risk factor for human carriage of ESBL. Among the eight ESBL-producing *E. coli* isolates from farm workers, the dominant $bla_{\text{CTX-M}}$ type was $bla_{\text{CTX-M-14}}$ (75.0%), followed by $bla_{\text{CTX-M-55}}$ (25.0%). $bla_{\text{CTX-M-14}}$ is distributed in both human and animal ESBL-producing *E. coli* isolates worldwide. In addition, several studies have shown that $bla_{\text{CTX-M-55}}$ in human ESBL-producing *E. coli* isolates has become the second most common CTX-M enzyme in China *(12, 24)*.

In summary, food animals can become reservoirs for ESBL-producing bacteria. Farm workers seem to have a higher risk for carrying ESBLs than the general population due to the fact that they have more chances to contact animals carrying ESBL-producing bacteria.

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