

Isolation and Molecular Characterization of Multidrug-Resistant *Enterobacteriaceae* Strains from Pork and Environmental Samples in Xiamen, China

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ABSTRACT

This study was conducted to investigate the prevalence and molecular characterization of multidrug-resistant (MDR) *Enterobacteriaceae* isolated from swine meat and the breeding environment. A total of 102 MDR *Enterobacteriaceae* strains belonging to five genera were obtained from 210 samples collected from a large-scale swine farm from March 2012 to June 2013 in Xiamen, People's Republic of China. Among these MDR isolates, *Escherichia coli* strains were found most frequently in both meat and environmental samples, followed by *Citrobacter* spp., *Klebsiella* spp., and *Shigella* spp. The neighbor-joining phylogenetic tree indicated that 70.3% of *Escherichia* and 50% of *Citrobacter* isolates from meat samples shared 100% homology with relevant isolates from environmental samples. Resistance was most frequently observed to sulfonamide, trimethoprim, aminoglycoside, chloramphenicol, β-lactam, and tetracycline. Close correlation was noted between antibiotic resistance phenotype and the genes responsible for resistance to sulfonamide (*sull*), trimethoprim (*dhfr1*), aminoglycoside (*aadA*, *aac(3)-I*, *aphA-1*, and *aac(3)-IV*), chloramphenicol (*catI* and *cmlA*), β-lactam (*blaSHV*, *blaOXA*, and *blaTEM*), florfenicol (*floR*), and tetracycline (*tet(A)* and *tet(B)*), which were widely distributed with prevalences of 72.5, 6.9, 62.7, 14.7, 78.4, 11.8, 25.5, 42.2, 12.7, 14.7, 39.2, 87.2, 68.6, and 34.3%, respectively. Class 1 integrons carrying *aadA22*, *dfrA17-aadA5*, or *dfrA12-aadA2* cassette arrays were commonly found in isolates from all samples. The gene cassette *aac(6')-Ib-cr-arr-3-dfrA27-aadA16* was first found in an *Enterobacter amnigenus* isolate. Conjugation experiments revealed the plasmid-mediated transfer of class 1 integrons. Our results indicate that swine meat and the farming environment can be sources of antibiotic-resistant bacteria, which could be potentially transmitted to humans via the meat products industry chain.

The increasing prevalence of antibiotic-resistant (ART) foodborne bacteria has become a public health concern. The emergence and spread of antibiotic resistance (AR) among bacteria from food animal sources is mainly caused by use of antibiotics in animal husbandry (5, 18). In the past few decades, antibiotics have been widely used in veterinary medicine for animal disease treatment or as growth promoters all over the world (1, 5, 36, 50). As the largest producer and consumer of antibiotics in the world, People's Republic of China produced 210 million kg of antibiotics each year, and 46.1% were used in the livestock industries (15). Approximately 75% of the antibiotics applied are excreted unaltered and then discharged into the environment (46). As a consequence, food animals (e.g., swine, cattle, turkey, chickens, and ducks) and the farm environment are reservoirs for ART bacteria, especially the ART *Enterobacteriaceae* bacteria (2, 12, 21, 28, 37, 43). These ART

foodborne bacteria could be transmitted to humans via the food chain (8, 13, 25, 35).

As a particular concern, multidrug-resistant (MDR) resistant to at least three different classes of antimicrobials) foodborne bacteria, which may potentially increase the healthcare costs and the economic burden to families and societies, have been isolated from various sources, including food animals (11, 12, 43). Resistance to antibiotics in bacteria is mediated by complex mechanisms, and horizontal gene transfer is considered an important factor in the spread of AR (16). The main mobile genetic elements that can carry resistance-encoding genes are plasmids, integrons, transposons, and bacteriophages (16). Previous studies indicated that the transfer of plasmids carrying class 1 integrons with inserted gene cassettes might be the main mechanism for the rapid spread of multidrug resistance in bacteria, especially among the *Enterobacteriaceae* (5, 40).

The *Enterobacteriaceae* is a large family of gram-negative bacteria. Some species in this family are infectious and cause serious health problems to consumers. The emergence of ART *Enterobacteriaceae* is a significant

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problem that requires particular attention, especially the occurrence of MDR strains. Although some kinds of MDR *Enterobacteriaceae* have been isolated from various types of samples, few comparative analyses of the MDR *Enterobacteriaceae* isolates from swine meat and the swine breeding environment have been reported.

The objective of this study was to analyze the homology of MDR *Enterobacteriaceae* isolates from swine meat and the swine breeding environment and the distribution of AR and resistance genes in these strains. The characteristics and transferability of class 1 integrons were examined to evaluate the spread of MDR *Enterobacteriaceae* strains and AR genes in the meat products industry chain.

MATERIALS AND METHODS

Bacterial isolates. A total of 210 fresh swine meat and environmental samples were obtained from a large-scale swine farm from March 2012 to June 2013. At our testing farm, sulfamethoxazole-trimethoprim, tetracycline, gentamicin, streptomycin, florfenicol, and amoxicillin were widely used for the treatment of swine infections or as growth promoters. Meat samples (about 200 g) were rinsed with 50 ml of sterile peptone water. About 20 g of each sample was aseptically placed in sterile lateral filter bags containing 20 ml of 0.1% sterile peptone water, and the following procedures were performed as described previously (17, 52, 54). Environmental samples (swine feces, swine farm wastewater, and soil samples) were collected by using a bacteria sampling tube (Youkang Biological Technology Co., Ltd., Beijing, People's Republic of China). The homogenized samples and rinsing liquids were serially diluted in sterile peptone water and plated on brain heart infusion (BHI; HKM, Guangzhou, People's Republic of China) agar plates containing 512 µg ml⁻¹ sulfamethoxazole (Sangon, Shanghai, People's Republic of China) and 16 µg ml⁻¹ tetracycline (Sigma, St. Louis, MO). All plates contained 100 µg ml⁻¹ cycloheximide (Sigma) and were incubated at 36 ± 1°C for 48 h. For each sample, morphologically different colony types were spotted on BHI agar plates supplemented with 16 µg ml⁻¹ trimethoprim (Sigma), 32 µg ml⁻¹ ampicillin (Sigma), 32 µg ml⁻¹ chloramphenicol (Sigma), 32 µg ml⁻¹ streptomycin (Sigma), or 256 µg ml⁻¹ fosfomycin (Sigma) for rapid profiling of MDR bacteria. Isolates that grew on two or more antibiotic-containing plates were chosen for further research.

Identification of bacterial isolates. The isolates were first identified with 16S rRNA amplification. The genomic DNA of every isolate was extracted by using the Bacterial Genomic DNA Extraction Kit (Sangon) according to the manufacturer's instructions. The 16S rRNA gene was amplified from the genomic DNA of the isolate by PCR using primers 27F (5'-AGAGTTT-GATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTGACTT-3') (22) and sequenced at the Sangon Biological Engineering Technology & Service Company (Shanghai). PCR conditions were as follows: 95°C for 5 min; 30 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 1.5 min; and then 72°C for 10 min. Homology was analyzed by comparison with GenBank sequences using the BLAST program. The target strains were further confirmed by using the Microbial Biochem Identification Tube System (HKM). MEGA 4.1 (Center for Evolutionary Functional Genomics, Tempe, AZ) was used to construct the neighbor-joining phylogenetic tree.

Antimicrobial susceptibility testing. The antimicrobial susceptibility of the isolates obtained from various sources was

determined using the disk diffusion method as described by the Clinical and Laboratory Standards Institute (7). The following 24 antibiotics were used: ampicillin (AMP, 10 µg), amoxicillin-clavulanic acid (AMC, 20 and 10 µg), cephalothin (CEF, 30 µg), cefotaxime (CTX, 30 µg), ceftizoxime (ZOX, 30 µg), cefoxitin (FOX, 30 µg), ceftazidime (CAZ, 30 µg), cefuroxime (CXM, 30 µg), cefamandole (MA, 30 µg), aztreonam (ATM, 30 µg), imipenem (IMP, 10 µg), gentamicin (GEN, 10 µg), kanamycin (KAN, 30 µg), streptomycin (STR, 10 µg), tetracycline (TET, 30 µg), doxycycline (DO, 30 µg), ciprofloxacin (CIP, 5 µg), norfloxacin (NOR, 10 µg), nalidixic acid (NAL, 30 µg), sulfamethoxazole (SMZ, 300 µg), trimethoprim (TMP, 5 µg), chloramphenicol (CHL, 30 µg), fosfomycin (FOS, 200 µg), and nitrofurantoin (F, 300 µg). *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213 were used as quality control strains.

PCR detection of resistance genes, integrons, and gene cassettes. Twenty-five AR genes were analyzed by using a multiplex PCR assay. Sets 1 through 5 were designed to detect the AR genes (30, 51). Set 1 contained primers for *sull* (0.56 µM), *blasHV* (0.56 µM), *catl* (0.28 µM), *dhfrV* (0.28 µM), *floR* (0.28 µM), *aadA* (0.28 µM), and *blaOXA* (0.28 µM). Set 2 contained the primers for *blaTEM* (0.56 µM), *cmlA* (0.28 µM), *citM* (0.28 µM), *ereA* (0.28 µM), *dhfrI* (0.28 µM), and *aac(3)-I* (0.56 µM). Set 3 contained primers for *aphA-1* (0.28 µM), *moxM* (0.28 µM), *dhaM* (0.28 µM), *ebcM* (0.28 µM), *aac(3)-IV* (0.28 µM), and *foxM* (0.28 µM). Set 4 contained the primers for *tet(B)* (0.25 µM), *tet(C)* (0.25 µM), and *tet(D)* (1.0 µM), and set 5 contained the primers for *tet(A)* (0.5 µM), *tet(E)* (0.5 µM), and *tet(G)* (0.5 µM). Multiplex PCR was carried out using the Multiplex PCR Assay Kit (Takara, Dalian, People's Republic of China). PCRs for multiplex sets were performed in a total volume of 50 µl including 1 µl of extracted DNA, 25 µl of Multiplex PCR Mix 2, 0.25 µl of Multiplex PCR Mix 1, and double-distilled water. PCR conditions for multiplex sets were as follows: 94°C for 5 min; 30 cycles of 94°C for 1 min, 58°C (set 4 and 5 were 55°C) for 1 min, and 72°C for 1.5 min; and then 72°C for 10 min. The presence of a class 1 integron was analyzed by uniplex PCR assay using previously described primers (21). Gene cassettes within the variable region of the class 1 integron were amplified with the primers 5'CS (5'-GGCATCCAAGCAGCAAG-3') and 3'CS (5'-AAGCAGACTTGACCTGA-3') as described previously (23). The PCR products were cloned into pMD-18T vectors (Takara) and sequenced at the Sangon Biological Engineering Technology & Service Company.

Conjugation experiments. The mating-out assay was performed using strains positive for the class 1 integron gene cassette with *E. coli* (20 isolates), *Shigella sonnei* (3 isolates), *Citrobacter* spp. (6 isolates), and *Klebsiella* spp. (3 isolates) as the donors and a rifampin-resistant strain of *E. coli* (strain RG488, a gift from Dr. Dong Taek Cho, Kyungpook National University, Daegu, Republic of Korea) as the recipient, as described previously (16, 27). Transforms were selected on BHI agar plates supplemented with rifampin (150 µg ml⁻¹; Sigma) and one of the following antimicrobials: streptomycin (50 µg ml⁻¹), trimethoprim (16 µg ml⁻¹), or chloramphenicol (50 µg ml⁻¹). Transfer of the class 1 integron was confirmed by PCR by using primers 5'CS and 3'CS.

RESULTS

Strain isolation and neighbor-joining phylogenetic tree construction. A total of 102 MDR *Enterobacteriaceae*

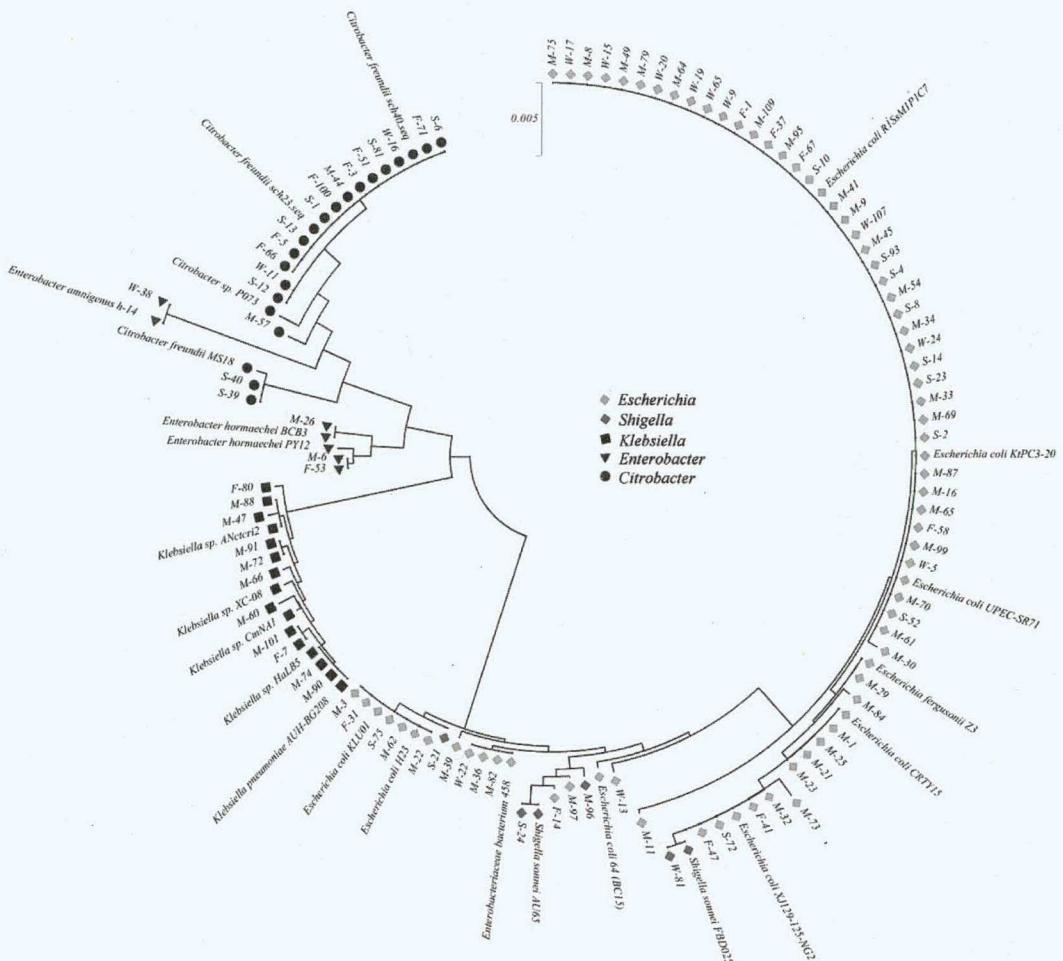


FIGURE 1. Neighbor-joining phylogenetic tree of the 102 MDR Enterobacteriaceae isolates from different types of samples. M, swine meat; F, swine feces; W, farm wastewater; S, farm soil. GenBank accession numbers: KF147134.1 (Escherichia coli RISSM1P1C7), KF025661.1 (E. coli KtPC3-20), KF192074.1 (E. coli UPEC-SR71), HQ259938.1 (Escherichia fergusonii Z3), KF574803.1 (E. coli CRYT15), JX975403.1 (E. coli XJ129-125-NG2), EU009199.1 (Shigella sonnei FBD02), KF254747.1 (E. coli 64 (BC15)), EF032687.1 (S. sonnei AU65), JN613165.1 (Enterobacteriaceae bacterium 458), JN129459.1 (E. coli H23), KC211290.1 (E. coli KLU01), GU128173.1 (Klebsiella pneumoniae AUH-BG208), HM352371.1 (Klebsiella sp. HalB5), HM352315.1 (Klebsiella sp. CmNA1), KC787534.1 (Klebsiella sp. XC-08), HQ286642.1 (Klebsiella sp. ANctcri2), KC759162.1 (Enterobacter hormaechei PY12), KF224906.1 (E. hormaechei BCB3), FN997616.1 (Citrobacter freundii MS18), KC139434.1 (Enterobacter amnigenus h-14), KC252813.1 (Citrobacter sp. P073), JX294897.1 (C. freundii sch23), JX294881.1 (C. freundii sch40).

isolates that grew on two or more antibiotic-containing plates were obtained from 210 fresh swine meat and environmental samples. Of these 102 isolates, 51 (at least eight species belonging to five genera) were isolated from fresh swine meat samples and 51 (at least seven species belonging to five genera) were isolated from environmental samples (17 from swine feces, 15 from farm wastewater, and 19 from farm soil) (Fig. 1). In general, *Escherichia* (72.5%) and *Klebsiella* (17.6%) were found more frequently in fresh swine meat samples, and *Escherichia* (56.9%) and *Citrobacter* (29.4%) were found more frequently in environmental samples. The neighbor-joining phylogenetic tree of the 102 MDR Enterobacteriaceae strains indicated that 26 of 37 *Escherichia* and 1 of 2 *Citrobacter* isolates from fresh swine meat samples shared 100% homology with relevant isolates from environmental samples. In other genera, the homologies of stains from meat and environmental samples were also high (Fig. 1).

Antimicrobial susceptibility testing. All 102 Enterobacteriaceae isolates were tested for sensitivity to the 24 antibiotics. Results revealed that 97 (95.1%) and 101 (99%) of the isolates were resistant to sulfamethoxazole and tetracycline, respectively (Table 1), and all isolates had multidrug resistance phenotypes. Among isolates obtained from meat samples, high frequencies of resistance were found to gentamicin (98% of isolates), streptomycin (98%), tetracycline (98%), chloramphenicol (98%), kanamycin (96.1%), doxycycline (96.1%), ciprofloxacin (94.1%), sulfamethoxazole (94.1%), trimethoprim (94.1%), norfloxacin (92.2%), nalidixic acid (78.4%), ampicillin (64.7%), and amoxicillin-clavulanic acid (60.8%). Among the isolates from environmental samples, widespread resistance was detected to tetracycline (100% of isolates), trimethoprim (100%), chloramphenicol (100%), doxycycline (96.1%), sulfamethoxazole (96.1%), streptomycin (94.1%), nalidixic acid (94.1%), gentamicin (92.1%), kanamycin (90.2%),

TABLE 1. Distribution of antibiotic resistance among the 102 MDR Enterobacteriaceae isolates

Antibiotic class	Abbreviation/type	No. (%) of isolates resistant to antimicrobial agents				
		Swine meat (n = 51)	Swine feces (n = 17)	Farm wastewater (n = 15)	Farm soil (n = 19)	Total (n = 102)
Sulfonamide	SMZ	48 (94.1)	16 (94.1)	15 (100)	18 (94.7)	97 (95.1)
Trimethoprim	TMP	48 (94.1)	17 (100)	15 (100)	19 (100)	99 (97.1)
Aminoglycoside	GEN	50 (98)	17 (100)	12 (80)	18 (94.7)	97 (95.1)
	KAN	49 (96.1)	16 (94.1)	12 (80)	18 (94.7)	95 (93.1)
	STR	50 (98)	17 (100)	13 (86.7)	18 (94.7)	98 (96.1)
Chloramphenicol	CHL	50 (98)	17 (100)	15 (100)	19 (100)	101 (99)
β-Lactams	AMP	33 (64.7)	11 (64.7)	9 (60)	8 (42.1)	61 (59.8)
	AMC	31 (60.8)	15 (88.2)	8 (53.3)	10 (52.6)	64 (62.7)
	CEF	19 (37.3)	9 (52.9)	5 (33.3)	10 (52.6)	43 (42.2)
	CTX	9 (17.6)	1 (5.9)	3 (20)	7 (36.8)	20 (19.6)
	ZOX	2 (3.9)	1 (5.9)	1 (6.7)	6 (31.6)	10 (9.8)
	FOX	8 (15.7)	5 (29.4)	1 (6.7)	4 (21.1)	18 (17.6)
	CAZ	2 (3.9)	0	1 (6.7)	4 (21.1)	7 (6.9)
	CXM	11 (21.6)	4 (23.5)	4 (26.7)	9 (47.4)	28 (27.5)
	MA	10 (19.6)	2 (11.8)	5 (33.3)	9 (47.4)	26 (25.5)
	ATM	5 (9.8)	1 (5.9)	1 (6.7)	6 (31.6)	13 (12.7)
	IMP	6 (11.8)	3 (17.6)	2 (13.3)	5 (26.3)	16 (15.7)
Tetracycline	TET	50 (98)	17 (100)	15 (100)	19 (100)	101 (99)
	DO	49 (96.1)	16 (94.1)	14 (93.3)	19 (100)	98 (96.1)
Others	CIP	48 (94.1)	11 (64.7)	10 (66.7)	15 (78.9)	84 (82.4)
	NOR	47 (92.2)	11 (64.7)	9 (60)	14 (73.4)	81 (79.4)
	NAL	40 (78.4)	14 (82.4)	15 (100)	19 (100)	88 (86.3)
	FOS	8 (15.7)	2 (11.8)	3 (20)	0	13 (12.7)
	F	21 (41.2)	9 (52.9)	3 (20)	1 (5.3)	34 (33.3)

ciprofloxacin (70.5%), norfloxacin (66.7%), amoxicillin-clavulanic acid (64.7%), and ampicillin (54.9%) (Table 1). Few isolates from both meat and environmental samples were resistant to ceftizoxime, ceftazidime, aztreonam, imipenem, and fosfomycin. No isolates from swine feces were resistant to ceftazidime, and no isolates from farm soil samples were resistant to fosfomycin (Table 1). High levels of norfloxacin and ciprofloxacin resistance were found among *Escherichia* and *Klebsiella* strains from both meat and environmental samples, but resistance to these antimicrobials was found more frequently in swine meat isolates (Table 2). Almost all isolates with the characteristics of MDR were resistant to sulfamethoxazole, trimethoprim, tetracycline, gentamicin, streptomycin, and chloramphenicol.

Detection of resistance genes. The prevalence of 25 resistance genes in 102 *Enterobacteriaceae* isolates was determined with a multiplex PCR assay. The PCR results were closely correlated with AR phenotype and isolate genotype (Tables 1 through 3). In total, genes responsible for resistance to sulfonamide (*sull*), trimethoprim (*dfr1*), aminoglycoside (*aadA*, *aac(3)-I*, *aphA-1*, and *aac(3)-IV*), chloramphenicol (*catI* and *cmlA*), β-lactam (*blaSHV*, *blaOXA*, and *blaTEM*), florfenicol (*floR*), and tetracycline (*tet(A)* and *tet(B)*) were widely distributed with rates of 72.5, 6.9, 62.7, 14.7, 78.4, 11.8, 25.5, 42.2, 12.7, 14.7, 39.2, 87.2, 68.6, and 34.3%, respectively. In contrast, genes *dfrV*, *citM*, *moxM*, *ebcM*, *foxM*, *tet(C)*, *tet(D)*, *tet(E)*, and *tet(G)* were not detected in any isolates (Table 3).

Four kinds of *ampC* resistance genes were detected in this research, and the *dhaM* gene was detected in isolates only from swine meat samples. However, all *ampC* resistance genes were not observed in isolates from environmental samples. As for the *dhaM* gene, the *ereA* gene conferring resistance to erythromycin was found only in isolates from swine meat samples.

Among tetracycline resistance genes, only *tet(A)* and *tet(B)* were found in tested isolates. One isolate (*E. coli* W-17) from a swine farm wastewater sample and two isolates (*Shigella sonnei* S-21 and *Citrobacter freundii* S-81) from farm soil samples contained both the *tet(A)* and *tet(B)* genes (Table 2).

Characteristics and transfer of class 1 integrons.

Ninety-two percent of isolates were positive for class 1 integrons. The class 1 integron genes were detected in 94 and 90% of the swine meat and environmental isolates, respectively. Among the isolates positive for class 1 integrons, 92.6% carried resistance gene cassettes (data not shown).

Resistance gene cassettes *aadA22*, *dfrA17-aadA5*, and *dfrA12-aadA2* were most frequently found in *Escherichia* and *S. sonnei* isolates from both swine meat and environmental samples (Table 2). All *Citrobacter* isolates positive for class 1 integrons carried at least one resistance gene cassette; the cassettes *dfrA17-aadA5* and *dfrA12-aadA2* were most frequently observed in these isolates. Among the *Klebsiella* isolates, three kinds of gene cassettes (*arr-3*-

TABLE 2. Summary of antibiotic resistance profiles and the presence of antibiotic resistance genes and gene cassettes in representative isolates

Isolate name	Food source	Antibiotic resistance characteristics		
		Pattern	Genes	Gene cassette(s)
<i>Escherichia coli</i>				
M-1	Meat	AMP, CEF, GEN, KAN, STR, TET, DO, NAL, SMZ, CHL	<i>floR</i> , <i>aadA</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(B)</i>	<i>aadA22</i>
M-21	Meat	AMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>aadA22</i>
M-22	Meat	CEF, CTX, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>floR</i> , <i>aadA</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(B)</i>	<i>dfrA17-aadA5</i> , <i>aadA22</i>
M-23	Meat	AMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>dfrA12-aadA2</i> , <i>aadA22</i>
M-30	Meat	AMP, AMC, GEN, KAN, STR, TET, DO, CIP, NOR, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	ND ^a
M-32	Meat	AMP, CEF, GEN, KAN, STR, TET, DO, CIP, NOR, SMZ, TMP, CHL	<i>sull</i> , <i>catI</i> , <i>floR</i> , <i>aadA</i> , <i>blaTEM</i> , <i>tet(A)</i>	<i>aadA22</i>
M-39	Meat	GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>blaOXA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>aadA22</i>
M-70	Meat	AMP, AMC, CEF, CXM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>floR</i> , <i>aadA</i> , <i>blaOXA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>aac(3)-IV</i> , <i>tet(A)</i>	<i>aadA2-linF</i>
M-73	Meat	CEF, CTX, CXM, MA, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(B)</i>	<i>aacA4-cmlA1</i>
M-95	Meat	AMP, AMC, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	<i>sull</i> , <i>floR</i> , <i>aadA</i> , <i>blaTEM</i> , <i>dhfrI</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>dfrA17-aadA5</i> , <i>aadA22</i>
M-97	Meat	AMP, AMC, CEF, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aac(3)-I</i> , <i>tet(A)</i>	ND
S-4	Soil	AMP, AMC, CEF, FOX, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>catI</i> , <i>floR</i> , <i>aadA</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>aadA22</i>
S-8	Soil	AMP, AMC, CEF, CTX, ZOX, CAZ, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>aadA</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>dfrA12-aadA2</i> , <i>aadA22</i>
S-14	Soil	GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>catI</i> , <i>floR</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(B)</i>	<i>dfrA12-aadA2</i>
S-72	Soil	AMP, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>aadA22</i>
S-75	Soil	AMP, AMC, CEF, CTX, ZOX, CAZ, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>floR</i> , <i>blaOXA</i> , <i>cmlA</i> , <i>tet(A)</i>	<i>aadB-orf1-cmlA</i> , <i>dfrA17-aadA5</i>
W-17	Water	AMP, GEN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>catI</i> , <i>floR</i> , <i>blaTEM</i> , <i>tet(B)</i> , <i>tet(A)</i>	<i>dfrA12-aadA2</i>
W-24	Water	GEN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aac(3)-IV</i> , <i>tet(B)</i>	<i>dfrA12-aadA2</i>
W-65	Water	AMP, AMC, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>catI</i> , <i>aadA</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>dfrA17-aadA5</i> , <i>aadA22</i>
W-107	Water	GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(B)</i>	<i>aadA22</i>
F-1	Feces	AMP, AMC, CEF, CTX, ZOX, CXM, MA, ATM, IMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, FOS	<i>sull</i> , <i>catI</i> , <i>aadA</i> , <i>blaOXA</i> , <i>dhfrI</i> , <i>aac(3)-IV</i> , <i>tet(A)</i>	<i>aadA1</i>
F-14	Feces	AMP, AMC, CEF, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aac(3)-IV</i> , <i>tet(A)</i>	<i>aac(6')-Ib-cr-arr-3</i> , <i>dfrA27-aadA16</i>
F-31	Feces	AMP, AMC, CEF, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>floR</i> , <i>aadA</i> , <i>blaOXA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>aac(3)-IV</i> , <i>tet(A)</i>	<i>aadA22</i>
F-37	Feces	AMP, AMC, GEN, KAN, STR, TET, DO, CIP, NOR, SMZ, TMP, CHL, F	<i>sull</i> , <i>floR</i> , <i>aadA</i> , <i>dhfrI</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>dfrA17-aadA5</i> , <i>aadA22</i>
F-41	Feces	AMP, CEF, CTX, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	<i>sull</i> , <i>catI</i> , <i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>aadA22</i>
F-58	Feces	AMC, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	<i>sull</i> , <i>catI</i> , <i>floR</i> , <i>aadA</i> , <i>blaTEM</i> , <i>aphA-1</i> , <i>tet(A)</i>	ND

TABLE 2. *Continued*

Isolate name	Food source	Antibiotic resistance characteristics		
		Pattern	Genes	Gene cassette(s)
F-67	Feces	GEN, KAN, STR, TET, DO, CIP, NOR, NAL, TMP, CHL, F	sull, floR, aadA, cmlA, apha-I, tet(A)	aadA22
<i>E. fergusonii</i>	Meat	GEN, KAN, STR, TET, DO, CIP, NOR, NOR, SMZ, TMP, CHL	floR, aadA, cmlA, apha-I, tet(B)	aadA22
	Meat	GEN, KAN, STR, TET, DO, CIP, NOR, SMZ, TMP, CHL, F	floR, aadA, cmlA, apha-I, tet(B)	aadA22
	Meat	AMC, GEN, KAN, STR, TET, DO, CIP, NOR, SMZ, TMP, CHL	floR, aadA, cmlA, apha-I, tet(B)	aadA22
	Water	AMP, AMC, CEF, CTX, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	floR, aadA, blaOXA, cmlA, apha-I, tet(A)	aadA22
<i>Escherichia</i> sp.	Meat	AMP, AMC, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	floR, aadA, blaOXA, cmlA, apha-I, tet(B)	dfrA17-aadA5, aadA22
	Soil	AMP, GEN, KAN, STR, TET, DO, CIP, SMZ, TMP, CHL	sull, floR, aadA, dhfrI, apha-I, tet(A)	ND
	Soil	AMP, AMC, CEF, CTX, ZOX, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	sull, floR, aadA, cmlA, apha-I, tet(B), tet(A)	dfrA27-arr-3
	Soil	AMP, AMC, CEF, CTX, ZOX, CAZ, CXM, MA, ATM, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, catI, blaTEM, tet(A)	dfrA12-aadA2, dfrA17-aadA5
<i>Shigella sonnei</i>	Water	AMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	floR, aadA, cmlA, apha-I, tet(A)	aadA22
	Meat	AMP, GEN, KAN, STR, TET, DO, CIP, SMZ, TMP, CHL	sull, floR, aadA, dhfrI, apha-I, tet(A)	dfrA17-aadA5, aadA22
	Soil	AMP, AMC, CEF, CTX, ZOX, CXM, MA, ATM, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	sull, floR, aadA, cmlA, apha-I, tet(B), tet(A)	dfrA12-aadA2
	Soil	AMP, AMC, CEF, CTX, ZOX, CAZ, CXM, MA, ATM, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, catI, blaTEM, tet(A)	dfrA17-aadA5
<i>Citrobacter freundii</i>	Meat	AMP, AMC, CEF, FOX, MA, IMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	sull, catI, ereA, tet(A)	dfrA12-aadA2
	Soil	AMP, FOX, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, floR, blaTEM, aac(3)-I, apha-I, tet(B)	dfrA17-aadA5
	Soil	FOX, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, floR, blaTEM, aac(3)-I, tet(A)	dfrA12-aadA2
	Soil	GEN, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	sull, catI, floR, blaTEM, aac(3)-I, tet(A)	dfrA12-aadA2
<i>Citrobacter</i> sp.	Soil	CEF, FOX, IMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	sull, floR, blaTEM, aac(3)-I, apha-I, tet(A)	dfrA12-aadA2
	Soil	IMP, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, floR, blaTEM, aac(3)-I, apha-I, aac(3)-IV, tet(B), tet(A)	dfrA12-aadA2
	Water	AMP, AMC, FOX, IMP, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, floR, blaTEM, aac(3)-I, tet(A)	dfrA12-aadA2
	Water	AMP, FOX, IMP, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, blaSHV, floR, blaTEM, aac(3)-I, tet(A)	dfrA17-aadA5
F-3	Feces	AMC, CEF, IMP, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL, F	sull, floR, blaTEM, aac(3)-I, apha-I, tet(B), tet(A)	dfrA17-aadA5
F-51	Feces	AMC, FOX, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL, F	sull, catI, blaTEM, aac(3)-I, apha-I, tet(B)	dfrA17-aadA5, aadA22
F-66	Feces	AMC, AMC, FOX, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, catI, blaTEM, aac(3)-I, apha-I, tet(B)	dfrA17-aadA5
F-100	Feces	AMC, AMC, FOX, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	sull, catI, aadA, blaTEM, aac(3)-I, apha-I, tet(A)	dfrA17-aadA5
M-57	Meat	AMC, GEN, KAN, STR, TET, DO, NAL, SMZ, TMP, CHL	sull, floR, aadA, ereA, dhfrI, tet(A)	dfrA1-aadA1

TABLE 2. *Continued*

Isolate name	Food source	Antibiotic resistance characteristics		
		Pattern	Genes	Gene cassette(s)
<i>Klebsiella</i> sp.				
M-47	Meat	AMP, AMC, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	<i>sull</i> , <i>blaSHV</i> , <i>floR</i> , <i>blaOXA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>arr-3-dfrA27</i>
M-60	Meat	AMP, AMC, CEF, FOX, IMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, FOS, F	<i>sull</i> , <i>blaSHV</i> , <i>floR</i> , <i>aphA-1</i> , <i>dhaM</i> , <i>tet(A)</i>	<i>aac(6')-Ib-cr-arr-3-dfrA27-aadA16</i>
M-90	Meat	AMP, AMC, FOX, GEN, KAN, STR, TET, DO, CIP, NOR, SMZ, TMP, CHL	<i>sull</i> , <i>catI</i> , <i>floR</i> , <i>aadA</i> , <i>cmlA</i> , <i>aphA-1</i> , <i>tet(A)</i>	<i>aadA22</i>
M-101	Meat	AMP, AMC, CEF, ZOX, FOX, IMP, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ; TMP, CHL, FOS, F	<i>sull</i> , <i>blaSHV</i> , <i>floR</i> , <i>aphA-1</i> , <i>dhaM</i> , <i>tet(A)</i>	<i>aac(6')-Ib-cr-arr-3-dfrA27-aadA16</i>
F-7	Feces	AMC, CEF, CXM, MA, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>floR</i> , <i>aphA-1</i> , <i>tet(A)</i>	ND
F-80	Feces	AMP, AMC, GEN, KAN, STR, TET, DO, CIP, NOR, NAL, TMP, CHL, FOS, F	<i>sull</i> , <i>blaSHV</i> , <i>floR</i> , <i>blaOXA</i> , <i>aphA-1</i> , <i>tet(A)</i>	ND
<i>Enterobacter hormaechei</i>				
M-6	Meat	AMC, CEF, FOX, IMP, GEN, STR, TET, DO, SMZ, TMP, CHL, F	<i>sull</i> , <i>floR</i> , <i>blaTEM</i> , <i>tet(A)</i>	<i>dfrA12-aadA2</i>
M-26	Meat	AMP, AMC, CEF, FOX, CXM, MA, GEN, STR, TET, DO, CIP, NOR, NAL, SMZ, TMP, CHL, F	<i>sull</i> , <i>floR</i> , <i>blaTEM</i> , <i>tet(A)</i>	<i>dfrA12-aadA2</i>
F-53	Feces	AMP, AMC, CEF, FOX, IMP, GEN, STR, TET, DO, SMZ, TMP, CHL, F	<i>sull</i> , <i>floR</i> , <i>blaTEM</i> , <i>tet(B)</i> , <i>tet(A)</i>	<i>dfrA12-aadA2</i>
<i>E. amnigenus</i>				
W-38	Water	AMP, CEF, GEN, STR, TET, CIP, NAL, SMZ, TMP, CHL	<i>sull</i> , <i>catI</i> , <i>blaTEM</i> , <i>tet(A)</i>	<i>aac(6')-Ib-cr-arr-3-dfrA27-aadA16</i>

^a ND, not detected.

dfrA27, *aac(6')-Ib-cr-arr-3-dfrA27-aadA16*, and *aadA22*) were found in five isolates.

Other resistance gene cassettes such as *aadA2-linF*, *aacA4-cmlA1*, *aadB-orf1-cmlA*, *dfrA27-arr-3*, and *dfrA1-aadA1* were also found in some tested isolates (Table 2). Eleven isolates (eight *Escherichia*, two *S. sonnei*, and one *C. freundii*) were positive for two kinds of gene cassettes simultaneously; the combinations *dfrA12-aadA2* plus *aadA22* and *dfrA17-aadA5* plus *aadA22* were most frequently observed. The resistance gene cassette *aac(6')-Ib-cr-arr-3-dfrA27-aadA16* was first found in an *Enterobacter amnigenus* isolate.

The transferability of the class 1 integron was examined by conjugation experiments. The results revealed that four class 1 integrons (three carrying the *dfrA12-aadA2* array and one carrying the *adB-orf1-cmlA* array) in *E. coli* isolates from swine meat and environmental samples, one class 1 integron carrying the *dfrA12-aadA2* array in *S. sonnei* isolates from farm soil samples, and three class 1 integrons (two carrying the *dfrA12-aadA2* array and one carrying the *dfrA1-aadA1* array) in *Citrobacter* isolates from swine meat and environmental samples could horizontally transfer to *E. coli*. The rest of the class 1 integrons failed to transfer.

DISCUSSION

In this study, 102 MDR *Enterobacteriaceae* isolates were obtained from fresh swine meat and environmental samples from a large-scale swine farm where sulfonamide-

trimethoprim, aminoglycoside, and tetracycline antibiotics were widely used. Plates containing different antibiotics were used to select MDR isolates, and the antibiotic tolerance of selected isolates was further examined by antimicrobial susceptibility testing. Almost all selected isolates were resistant to three or more kinds of antibiotics, which indicated that this method was a fast and effective way to screen MDR isolates. Similar methods have been used previously to select ART bacterial isolates from various samples (31, 53, 57).

Among the 102 MDR *Enterobacteriaceae* bacteria isolates, *E. coli* was the most dominant MDR isolate in both meat and environmental samples, followed by *Citrobacter* and *Klebsiella*. *E. coli* strains exist extensively in various livestock meat and environments and often exhibit resistance to antibiotics (3, 46, 49, 51). Recently, several studies have been published concerning the antimicrobial resistance of *Citrobacter*, *Klebsiella*, and other *Enterobacteriaceae* strains isolated from meat and environmental samples (11, 12, 28, 43). In our study, the homologies of isolates of the same genus from meat and environmental samples were very high (Fig. 1), e.g., 70.3% of *Escherichia* and 50% of *Citrobacter* isolates from meat samples shared 100% homology with relevant isolates from environmental samples. Some of the isolates with high homology had similar AR phenotypes and contained the same AR genes and gene cassettes (Table 2). The results indicate that swine meat and the swine farming environment can be sources of ART bacteria, which could be

TABLE 3. Distribution of antibiotic resistance genes among the 102 MDR Enterobacteriaceae isolates

Antimicrobial resistance	Gene	No. (%) of isolates containing resistance genes				
		Swine meat (n = 51)	Swine feces (n = 17)	Farm wastewater (n = 15)	Farm soil (n = 19)	Total (n = 102)
Sulfonamide	<i>sull</i>	36 (70.6)	14 (82.4)	7 (46.7)	17 (89.5)	74 (72.5)
	<i>dhfrV</i>	0	0	0	0	0
Trimethoprim	<i>dhfrI</i>	3 (5.8)	3 (17.6)	0	1 (5.3)	7 (6.9)
	<i>aadA</i>	36 (70.6)	10 (58.8)	9 (60)	9 (47.4)	64 (62.7)
Aminoglycoside	<i>aac(3)-I</i>	4 (7.8)	4 (23.5)	1 (7)	6 (31.6)	15 (14.7)
	<i>aphA-1</i>	45 (88.2)	12 (70.6)	10 (66.7)	13 (68.4)	80 (78.4)
Chloramphenicol	<i>aac(3)-IV</i>	6 (11.8)	3 (17.6)	2 (13.3)	1 (5.3)	12 (11.8)
	<i>catI</i>	11 (21.6)	5 (29.4)	4 (26.7)	6 (31.6)	26 (25.5)
β -Lactam	<i>cmlA</i>	26 (51)	3 (17.6)	7 (46.7)	7 (36.8)	43 (42.2)
	<i>blaSHV</i>	9 (17.6)	1 (5.9)	1 (7)	2 (10.5)	13 (12.7)
	<i>blaOXA</i>	10 (19.6)	3 (17.6)	1 (7)	1 (5.3)	15 (14.7)
	<i>blaTEM</i>	13 (25.5)	7 (41.2)	8 (53.3)	12 (63.2)	40 (39.2)
AmpC	<i>citM</i>	0	0	0	0	0
	<i>moxM</i>	0	0	0	0	0
	<i>dhaM</i>	4 (7.8)	0	0	0	4 (3.9)
	<i>ebcM</i>	0	0	0	0	0
Macrolide	<i>ereA</i>	2 (3.9)	0	0	0	2 (1.9)
	<i>floR</i>	50 (98)	12 (70.6)	12 (80)	15 (78.9)	89 (87.2)
Florfenicol	<i>foxM</i>	0	0	0	0	0
	<i>tet(A)</i>	38 (74.5)	11 (64.7)	7 (46.7)	14 (73.7)	70 (68.6)
FOXM	<i>tet(B)</i>	13 (25.5)	6 (35.5)	9 (60)	7 (36.8)	35 (34.3)
	<i>tet(C)</i>	0	0	0	0	0
Tetracycline	<i>tet(D)</i>	0	0	0	0	0
	<i>tet(E)</i>	0	0	0	0	0
	<i>tet(G)</i>	0	0	0	0	0

potentially transmitted to humans via the meat products industry chain.

The results of the antimicrobial susceptibility tests indicated that the most prevalent antibiotic resistance was to sulfonamide, aminoglycoside, chloramphenicol, β -lactam, and tetracycline. The high frequency of resistance to these antibiotics in isolates from meat and environmental samples is probably due to the extended use of these antibiotics in pig breeding farms. In other studies, *Enterobacteriaceae* were isolated from swine meat samples from a slaughterhouse and were resistant to sulfamethoxazole-trimethoprim, streptomycin, doxycycline, and penicillins (43). Tetracycline-resistant *Enterobacteriaceae* strains have been routinely isolated from all farm animals in South Korea, where tetracycline has been widely used in veterinary medicine for the treatment of infections or as a growth promoter on farms (29, 38, 50). In Vietnam, Van et al. (51) found that *E. coli* isolates from raw meat had high rates of resistance to sulfonamide, tetracycline, ampicillin, aminoglycoside, and chloramphenicol, probably related to the lack of stringent controls on antibiotics usage in farm animals. In the United States, *E. coli* isolates from swine meat were most frequently resistant to tetracycline, sulfonamide, ampicillin, and streptomycin (42, 59). In contrast, few isolates survived on plates containing fosfomycin. Among the selected MDR strains, isolates from both meat and environmental samples had low levels of resistance to ceftizoxime, ceftazidime, aztreonam, imipenem, and fosfomycin (Table 1), which can most probably be attributed to the fact that these

antimicrobial agents were not used on the tested swine farm. In addition, isolates from meat samples had an AR distribution similar to that of strains from environmental samples (Table 1). Strains that were resistant to more than 15 antibiotics were found in all samples; among them, environmental isolates S-8, S-75, and F-1 were resistant to 20 or more antibiotics (Table 2). Our study indicates that the prudent use of antibiotics in farm animals is essential to limit the spread of ART bacteria.

Genes responsible for various AR characteristics were investigated by multiplex PCR. The genes conferring resistance to sulfonamide, trimethoprim, aminoglycoside, chloramphenicol, β -lactam, florfenicol, and tetracycline were widely distributed in all kinds of isolates, which and the presence of these genes was closely correlated with the AR phenotype. Among the sulfonamide-resistant isolates, 76.3% were positive for *sull* genes; this gene was also found in two of five sulfonamide-susceptible isolates. These results suggest that the *sull* gene (encoding the dihydropteroate synthase enzyme) was the predominant sulfonamide resistance gene in our MDR *Enterobacteriaceae* isolates. In other studies, among sulfamethoxazole resistance genes, *sull* was also found at the highest frequency in *Enterobacteriaceae* isolates obtained from food animal sources (19, 26). Two trimethoprim resistance genes (*dhfrV* and *dhfrI*) were included in the present study. However, *dhfrV* was not detected, and *dhfrI* was found only at low rates in *Escherichia* and *Citrobacter* isolates, which suggests that the high level of trimethoprim resistance was

probably conferred by the other trimethoprim resistance genes or integrons.

Four aminoglycoside resistance genes (*aadA*, *aac(3)-I*, *aphA-1*, and *aac(3)-IV*) were detected in isolates from all samples, and these genes were frequently found in aminoglycoside-resistant *Enterobacteriaceae* strains from food animal sources (10, 14, 26, 41, 51). Two genes (*catI* and *cmlA*) conferring resistance to chloramphenicol were also widely distributed in our tested strains. Of three β -lactam resistance genes, the *blaTEM* gene was found more frequently than the other two genes. Our findings are in agreement with those reported by other researchers (16, 26, 45). The narrow-spectrum penicillinase TEM-1 is common in animals (24); consequently, the detection of a high prevalence of ampicillin-resistant and *blaTEM*-positive isolates in our study was expected. The *blaTEM* gene was observed more frequently in *C. freundii* and *Enterobacter* strains than in the other species in this study.

Plasmids encoding AmpC enzymes often confer resistance to most β -lactam antibiotics, and spread of plasmid-mediated *ampC* β -lactamase genes between organisms has been documented (32, 34, 51, 56). In this study, among plasmid-mediated *ampC* genes, *dhaM* was detected in four *Klebsiella* isolates obtained from swine meat samples. The other three *ampC* genes were not detected in any isolates. Tetracycline resistance genes located on mobile elements have contributed to the rapid spread of tetracycline resistant (9, 47, 51). In our research, *tet(A)* was found most frequently among tetracycline-resistant isolates, followed by *tet(B)*. The *tet(A)* gene, which encodes an efflux protein, confers resistance to tetracycline but not to glycylcycline or minocycline (6, 39). In contrast, the efflux protein encoded by the *tet(B)* gene can confer resistance to tetracycline, minocycline, and doxycycline (26, 33). Consistent with our study, these two tetracycline genes have been reported as predominant in *Enterobacteriaceae* isolates from food animal sources (3, 44, 48, 51).

Class 1 integrons were detected in 94 of the 102 isolates, and 92.6% of these isolates were positive for at least one resistance gene cassette. The *aadA22*, *dfrA17-aadA5*, and *dfrA12-aadA2* cassette arrays were commonly found in isolates from all samples. These three cassette arrays have been found frequently in *Enterobacteriaceae* isolates from animal and environmental samples (4, 20, 46, 58), which suggests that class 1 integrons carrying gene cassettes play an important role in conferring resistance to trimethoprim and aminoglycoside among our MDR *Enterobacteriaceae* strains. Conjugation experiments revealed that some class 1 integrons carrying the *dfrA12-aadA2*, *dfrA1-aadA1*, or *aadB-orf1-cmlA* gene cassette array could horizontally transfer to *E. coli*, suggesting that these integrons are located on transmissible plasmids. Class 1 integrons carrying these gene cassette arrays located on transmissible plasmids have been found previously (55) (GenBank CP001856.1 and JN983048.1). Thus, the transmission of the plasmid harboring the class 1 integron may have accelerated the spread of the AR genes among bacterial species. The *aac(6')-Ib-cr-arr-3-dfrA27-aadA16* cassette array conferring resistance to aminoglycoside, rifampin, and trimethoprim also was

detected in several bacterial species (GenBank JF775514.1, GU165830.1, and EU675686.2), but to the best of our knowledge, this gene cassette array was first found in an *E. amnigenus* isolate.

In conclusion, this study provided data on distribution of AR and characteristics of various AR genes, class 1 integrons, and gene cassettes in MDR *Enterobacteriaceae* isolates obtained from a large-scale swine farm where antibiotics were widely used. As far as we know, this report is the first concerning isolation, homology comparison, and molecular characterization of MDR *Enterobacteriaceae* strains from swine meat and environmental samples collected from the same farm in the People's Republic of China. Our results indicated that the high frequency of AR in isolates from all samples is probably due to the extended use of antibiotics at the swine farm. The high level of homology found between strains from different samples and the presence of transmissible plasmids carrying class 1 integrons suggest that ART bacteria and genes can spread in the meat products industry chain, which may allow the transmission of the ART bacteria and genes through meat products to humans. Therefore, the reasonable use of antibiotics in animal husbandry is essential to limit the spread of ART bacteria and to protect public health.

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