International Journal of Biological, Life and Agricultural Sciences
ISSN: 2415-6612
Vol:5, No:12, 2011

Antibiotic Resistance Profile of Bacterial Isolates from Animal Farming Aquatic Environments and Meats in a Peri-Urban Community in South Korea

Hyunjin Rho , Bongjin Shin , Okbok Lee , Yu-Hyun Choi , Jiyoung Lee and Jaerang Rho

***Abstract*—**The increasing usage of antibiotics in the animal farming industry is an emerging worldwide problem contributing to the development of antibiotic resistance. The purpose of this work was to investigate the prevalence and antibiotic resistance profile of bacterial isolates collected from aquatic environments and meats in a peri-urban community in Daejeon, Korea. In an antibacterial susceptibility test, the bacterial isolates showed a high incidence of resistance (~ 26.04 %) to cefazolin, tetracycline, gentamycin, norfloxacin, erythromycin and vancomycin. The results from a test for multiple antibiotic resistance indicated that the isolates were displaying an approximately 5-fold increase in the incidence of multiple antibiotic resistance to combinations of two different antibiotics compared to combinations of three or more antibiotics. Most of the isolates showed multi-antibiotic resistance, and the resistance patterns were similar among the sampling groups. Sequencing data analysis of 16S rRNA showed that most of the resistant isolates appeared to be dominated by the classes *Betaproteobacteria* and *Gammaproteobacteria* in the phylum *Proteobacteria*.

***Keywords*—**Antibiotics, Antibiotic resistance, Antimicrobial resistance, Multi-resistance

I. Introduction

SINCE Alexander Fleming discovered penicillin, produced by *Penicillium notatum*, as the first antibiotic in modern medicine in 1928, antibiotics have been widely used for over 40 years in both humans and animals [1,2]. During recent decades especially, antibiotics have been extensively used in farm animals for the purposes of antimicrobial therapy, prophylaxis and growth promotion [3,4]. This increasing usage of antibiotics has led to a worldwide problem in the development of antibiotic resistance (AR) among bacterial populations during recent decades [1,2,3,4]. AR, initially a problem in hospital settings and associated with an increased number of nosocomial infections, has now broadly extended to humans, causing severe bacterial infections [1,4,5]. The emergence of AR in among humans has prompted concerns about the public health implications of antibiotic use in agriculture [3,4].Bacteria have developed resistances to all of the different classes of antibiotics discovered to date, and the most frequent type of resistance is acquired and transmitted horizontally via mobile genetic elements such as plasmids, transposons and integrons [1,2,6,7]. These mechanisms of obtaining resistance have resulted in the simultaneous development of resistance to several antibiotic classes, creating a very dangerous multiple antibiotic resistance (MAR) in bacterial strains [1,2,8]. The increase of MAR among bacterial populations is also due in part to their ability to acquire new antibiotic resistance genes from other resistant strains [1,2,8]. The prevalence of MAR in fecal samples or raw meats derived from farm animals has been reported [3,4,5]. The inappropriate antibacterial treatment and overuse of antibiotics for agricultural purposes, particularly for growth enhancement, have contributed to the increased prevalence of MAR in farm animals [3,4,5].

Most of the antibiotics used for agricultural purposes are only partially metabolized by animals and are then discharged through fecal contents either into sewage disposals or directly into rivers near animal farms [3,4]. Several kinds of bacterial species displaying MAR patterns, mainly *Escherichia coli* and enterococci, have been well documented in aquatic environments contaminated with animal fecal contents [9,10,11]. Consequently, the antibiotics used in agriculture are responsible for the increase in the prevalence of MAR in animal farming aquatic environments and may be directly linked to the AR problems in humans either via direct contact or through the food chain.

The current work investigated the prevalence of bacterial AR among samples collected from aquatic environments and supermarkets near animal farms and the samples' susceptibility to antibiotics. Three main issues were addressed: 1) the relationship between the degree of bacterial contamination and the prevalence of bacterial AR, 2) the resistance patterns in AR isolated bacteria, and 3) the phylogenetic composition of AR isolated bacteria. We report here a correlation between a high degree of bacterial contamination and the incidence of bacterial AR. Additionally, bacterial isolates showed a wide range of MAR patterns to seven or more antibiotics from ten kinds of antibiotics tested. Finally, we show that most of the resistant isolates appeared to be dominated by the classes *Betaproteobacteria and Gammaproteobacteria* in the phylum *Proteobacteria*.

[1]Daejeon Science High School, 19-2 Gusung-dong, Yuseong-gu, Daejeon 305-338, South Korea; [2]Department of Microbiology and Molecular Biology, [3]College of Education, and [4]GRAST, Chungnam National University, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, South Korea.

*Corresponding Author: Jaerang Rho, Ph.D. Department of Microbiology and Molecular Biology, 220 Gung-dong, Yuseong-gu, Daejeon 305-764, South Korea. Fax: 82-42-822-7367. Email: jrrho@cnu.ac.kr.

II. Materials and Methods

A. Sample collection and cultivations

Sampling sites were selected in animal farming regions or near vicinities located in a peri-urban community in Daejeon, Korea. Samples were collected from aquatic environments and markets near animal farms. The fecal-contaminated water samples were taken from the aquatic environments of the Sungwon pig farm (Water-PF) and the Gongam chicken farm (Water-CF). A water sample from Gongam river (Water-GR) was taken near an animal farm, and a Sangweol river sample (Water-SR) was taken near Mt. Gyeryong located away from animal farms. Water samples of fish tanks were obtained from the Gunsan sushi market (Water-SM) and the Kim fish market (Water-FM). Minced raw meat samples of pork (Meat-P) and chicken (Meat-C) were obtained from supermarkets. One gram of each meat sample was mixed with an equal volume of nutrient broth (Becton Dickinson Co., Sparks, MD,) and then the supernatant was used as a bacterial stock. All of the samples were diluted and processed for bacterial cultivation and isolation within 24 h. The colony forming unit (CFU) of cultured bacteria was calculated with bacterial cultivation on nutrient agar (NA) plate at 37℃ for 24 h.

B. Resistance to antibiotics

The NA plate sets contained one of the following ten individual antibacterial agents with different equivalent concentrations: cefazolin (CEF, 30 μg/ml), tetracycline (TET, 10 μg/ml), gentamicin (GEN, 10 μg/ml), norfloxacin (NOR, 10 μg/ml), erythromycin (ERY, 15 μg/ml), vancomycin (VAN, 6 μg/ml), doxycycline (DOX, 10 μg/ml), ampicillin (AMP, 30 μg/ml), kanamycin (KAN, 30 μg/ml), and chloramphenicol (CHL, 30 μg/ml). The plates were used to test single antibiotic resistance. Antibiotic doses were chosen based on a previous study by Kilonzo-Nthenge and colleagues [12]. For the MAR test, bacterial samples were cultured on multiple antibiotic-containing NA plates at 37℃ for 24 h, and then the resistant colony number was counted. The percentage of resistance of each sample was calculated as the ratio between the number of colonies on NA plates with and without antibiotics multiplied by 100.

C. Disc diffusion susceptibility test

The disc diffusion susceptibility test was performed as described previously [13,14]. Briefly, the resistant isolates were cultured in nutrient broth and incubated at 37℃ for 24 h under aerobic conditions, and then bacterial cultures were streaked evenly onto the entire surface of a NA plate and incubated at 37℃ for 24 h. The cultures were tested for sensitivity to ten antibiotics: CEF (30 μg/disk), TET (10 μg/disk), GEN (10 μg/disk), NOR (10 μg/disk), ERY (15 μg/disk), VAN (6 μg/disk), DOX (10 μg/disk), AMP (30 μg/disk), KAN (30 μg/disk), and CHL (30 μg/disk). The inhibition zones were measured and interpreted as susceptible (S), intermediate resistance (I) or resistant (R).

D. Minimum inhibitory concentration (MIC) test

For the MAR isolates, the MIC tests were performed as described previously [14]. A 50 μL aliquot of bacterial cultures (10^7 CFU/ml) from MAR isolates were inoculated into test tubes containing nutrient broth supplemented with different antibiotic concentrations. The test tubes were incubated at 37 ℃ for 16 h under aerobic conditions. Bacterial growth was visually detected by turbidity.

E. 16S rRNA sequence analysis and bacterial identification

The 16S rRNA sequence of the MAR isolates was analyzed from Macrogen Co., Korea. Briefly, the gene fragments of 16S rRNA were amplified by polymerase chain reaction (PCR), and the amplicons were sequenced by the bidirectional sequencing method. For the bacterial identifications, the 16S rRNA gene sequences were analyzed using the BLAST program (http://www.ncbi.nlm.nih.gov/BLAST/) to search in the GenBank database for the closet known relatives. The resultant 16S rRNA sequences were aligned together with corresponding sequences from representative species using the PHYDIT program (http://plaza.snu.ac.kr/~jchun/phydit) as described previously [15].

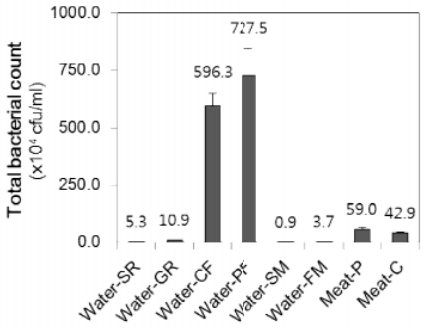

Fig. 1 Comparison of total bacterial count in different samples. Each count is an average of duplicate counts. The *t*-test for number of bacterial colony is significant ($p < 0.05$). Water-SR: Sutong river sample, Water-GR: Gap river sample, Water-CF: water sample from Dunggi chicken farm, Water-PF: water sample from Sungwon pig farm, Water-SM: water sample from Gunsan sushi market, Water-FM: water sample from Kim fish market, Meat-P: minced raw meat samples for the retailed pork, Meat-C: minced raw meat samples for the retailed chicken

III. Results

A. Resistance to antibiotics

We compared the total number of bacteria by calculating the colony forming unit (CFU) among collected samples in Fig. 1. The total number of bacterial colonies was significantly different, with values ranging from 9.0×10^3/ml to 7.3×10^6/ml depending on the sampling locations. The animal fecal-contaminated water samples collected directly from animal farms showed an approximately 55- to 137-fold increase in bacterial CFU than river water samples collected from a considerable distance from animal farms. In addition, the samples from the purchased raw pork and chicken showed a

significantly high number of bacterial CFU, indicating a high incidence of bacterial contamination. In contrast, we did not find any significant increase of bacterial CFU in water samples collected from fish tanks.

TABLE I
ANTIBIOTIC-RESISTANT BACTERIA DETECTED FROM DIFFERENT SAMPLES

Samples	CEF		TET		GEN		NOR		ERY		VAN	
	AR (n)	AR (%)	AR (n)	AR (%)	AR (n)	AR (%)	AR (n)	AR (%)	AR (n)	AR (%)	AR (n)	AR (%)
Water-SR	19.3×10^2	3.67	0	0	10.0×10^0	0.02	n.t.	n.t.	70.0×10^1	1.35	29.2×10^2	5.57
Water-GR	91.3×10^2	8.16	21.5×10^3	18.59	19.6×10^3	16.88	n.t.	n.t.	27.5×10^2	2.98	61.8×10^2	5.28
Water-CF	25.0×10^3	0.39	32.0×10^4	5.25	12.5×10^5	20.83	n.t.	n.t.	15.0×10^3	0.25	15.5×10^3	0.26
Water-PF	11.3×10^5	14.88	17.5×10^5	26.04	35.0×10^4	5.34	n.t.	n.t.	50.1×10^4	6.91	10.8×10^5	14.88
Water-SM	22.5×10^0	0.30	0	0	16.0×10^1	1.70	55.0×10^0	0.70	26.8×10^1	0.03	26.8×10^1	0.03
Water-FM	37.3×10^1	1.00	0	0	35.0×10^0	0.10	92.5×10^0	0.20	16.5×10^1	<0.01	13.5×10^1	<0.01
Meat-P	21.0×10^3	3.60	16.1×10^3	2.70	22.5×10^0	<0.01	13.1×10^2	0.70	13.8×10^1	<0.01	15.3×10^1	<0.01
Meat-C	30.4×10^3	4.70	64.4×10^2	1.50	44.9×10^2	1.00	19.2×10^3	4.50	23.3×10^1	<0.01	25.0×10^1	<0.01

Each count is an average of duplicate counts. n.t.: not tested. AR (n): colony forming unit (CFU/ml) in antibiotic resistance (AR). AR (%): the ratio of AR.

To compare the correlation between a high degree of bacterial contamination and the incidences of bacterial AR among the collected samples, we tested the antibacterial susceptibility with CEF, TET, GEN, NOR, ERY and VAN. We have shown the summary of AR among samples in Table 1. Most of the samples showed high resistances to single antibiotics tested. The animal fecal-contaminated water samples (Water-CF and -PF) showed relatively higher incidences of AR, up to ~ 26.04 %, than that of other tested samples (Table I). In addition, among individual antibiotics, the incidences of bacteria resistant to CEF (14.88 %), TET (26.04 %) and VAN (14.88 %) were higher than those of GEN (5.34 %) and ERY (6.91 %) in the pig fecal-contaminated water sample (Water-PF, Table 1). Subsequently, we showed a correlation between a high degree of bacterial contamination and the incidences of bacterial AR among collected samples.

TABLE II
MAR PROFILE OF RESISTANT ISOLATES FROM DIFFERENT SAMPLES

Samples	MAR profile	MAR (n)	MAR (%)	Samples	MAR profile	MAR (n)	MAR (%)
	CEF, GEN, TET	0	0		CEF, TET	205	2.28
	CEF, ERY, TET	0	0		CEF, NOR	148	1.64
	VAN, CEF, ERY	10	0.02		CEF, GEN	283	3.14
	GEN, CEF, ERY	0	0		TET, NOR	110	1.22
Water-SR	ERY, TET, VAN	0	0	Water-SM	TET, GEN	0	0
	GEN, VAN, TET	0	0		NOR, GEN	68	0.75
	VAN, CEF, TET	0	0		CEF, TET, NOR	118	1.31
	GEN, ERY, TET	0	0		CEF, GEN, TET	93	1.03
	GEN, ERY, VAN	0	0		TET, GEN, NOR	65	0.72
	VAN, CEF, GEN	0	0		TET, GEN, NOR, CEF	50	0.56
	CEF, GEN, TET	0	0		CEF, TET	15	0.04
	CEF, ERY, TET	0	0		CEF, NOR	225	0.61
	VAN, CEF, ERY	380	0.35		CEF, GEN	113	0.30
	GEN, CEF, ERY	5	0.01		TET, NOR	28	0.07
Water-GR	ERY, TET, VAN	5	0.01	Water-FM	TET, GEN	13	0.03
	GEN, VAN, TET	0	0		NOR, GEN	143	0.39
	VAN, CEF, TET	35	0.03		CEF, TET, NOR	5	0.01
	GEN, ERY, TET	0	0		CEF, GEN, TET	25	0.07
	GEN, ERY, VAN	0	0		TET, GEN, NOR	10	0.03
	VAN, CEF, GEN	5	0.01		TET, GEN, NOR, CEF	10	0.03
	CEF, GEN, TET	25	<0.01		CEF, TET	228	0.05
	CEF, ERY, TET	45	<0.01		CEF, NOR	180	0.04
	VAN, CEF, ERY	125	<0.01		CEF, GEN	160	0.04
	GEN, CEF, ERY	1280	0.02		TET, NOR	203	0.05
Water-CF	ERY, TET, VAN	220	<0.01	Meat-C	TET, GEN	155	0.04
	GEN, VAN, TET	115	<0.01		NOR, GEN	83	0.02
	VAN, CEF, TET	140	<0.01		CEF, TET, NOR	65	0.02
	GEN, ERY, TET	30	<0.01		CEF, GEN, TET	83	0.02
	GEN, ERY, VAN	15	<0.01		TET, GEN, NOR	50	0.01
	VAN, CEF, GEN	1635	0.03		TET, GEN, NOR, CEF	33	0.01
	CEF, GEN, TET	50	<0.01		CEF, TET	145	0.02
	CEF, ERY, TET	1310	0.02		CEF, NOR	78	0.01
	VAN, CEF, ERY	27090	0.37		CEF, GEN	88	0.01
	GEN, CEF, ERY	12375	0.17		TET, NOR	55	0.01
Water-PF	ERY, TET, VAN	9160	0.13	Meat-P	TET, GEN	55	0.01
	GEN, VAN, TET	125	<0.01		NOR, GEN	30	0.01
	VAN, CEF, TET	7645	0.11		CEF, TET, NOR	50	0.01
	GEN, ERY, TET	15	<0.01		CEF, GEN, TET	40	0.01
	GEN, ERY, VAN	55	<0.01		TET, GEN, NOR	35	0.01
	VAN, CEF, GEN	11770	0.16		TET, GEN, NOR, CEF	13	<0.01

Each count is an average of duplicate counts. MAR (n): colony forming unit (CFU/ml) in multiple antibiotic resistance (MAR). MAR (%): the ratio of MAR.

TABLE III
IDENTIFICATION AND RESISTANCE PATTERNS OF THE RESISTANT ISOLATES FROM DIFFERENT SAMPLES

Samples	Code : Species assignment (score in %)	TET	DOX	CEF	AMP	NOR	KAN	GEN	CHL	ERY	VAN
Water-SR	B1-1 : *Burkholderia cepacia* (100.0)	R	R	R	R	S	S	R	R	R	I
	B1-2 : *Delftia acidovorans* (98.7)	R	I	R	R	S	I	R	R	R	R
	B1-3 : *Pseudomonas alcaligenes* (98.8)	R	R	R	R	R	S	S	S	R	R
Water-GR	B2-1 : *Delftia lacustris* (100.0)	I	S	R	R	S	R	R	R	R	R
	B2-2 : *Shigella flexneri* (100.0)	R	I	I	R	S	S	I	S	R	R
	B2-3 : *Delftia lacustris* (100.0)	R	I	R	R	S	R	R	I	R	R
Water-CF	B3-1 : *Delftia lacustris* (100.0)	I	I	R	R	I	I	R	R	R	R
	B3-2 : *Enterobacter hormaechei* (99.6)	R	I	R	R	R	R	R	R	R	R
	B3-3 : *Enterobacter cancerogenus* (99.7)	R	I	R	R	S	S	I	S	R	R
Water-PF	B4-1 : *Delftia lacustris* (90.7)	S	I	R	R	I	S	I	S	R	R
	B4-2 : *Acinetobacter johnsonii* (98.5)	R	S	I	R	R	S	I	S	I	R
	B4-3 : *Pseudomonas plecoglossicida* (99.8)	R	I	R	R	S	S	S	R	R	R
Water-SM	B5-1 : *Escherichia coli* (99.9)	R	I	R	R	R	S	I	R	R	R
	B5-2 : *Escherichia coli* (98.5)	R	I	R	R	R	S	I	R	R	R
	B5-3 : *Pseudomonas hibiscicola* (99.8)	R	I	R	R	R	R	R	S	R	R
Water-FM	B6-1 : *Pseudomonas hibiscicola* (99.2)	I	I	R	R	R	R	R	R	R	R
	B6-2 : *Pseudomonas hibiscicola* (99.2)	R	I	R	R	R	R	R	R	R	R
	B6-3 : *Pseudomonas hibiscicola* (99.9)	S	I	S	I	S	S	S	S	S	S
Meat-C	B7-1 : *Escherichia coli* (99.9)	R	I	R	R	R	S	I	I	R	R
	B7-2 : *Escherichia coli* (99.9)	R	I	R	R	R	I	I	R	I	R
	B7-3 : *Pseudomonas hibiscicola* (99.9)	R	I	R	R	R	R	R	R	R	R
Meat-P	B8-1 : *Escherichia coli* (99.9)	R	I	R	R	R	I	I	I	R	R
	B8-2 : *Escherichia coli* (99.9)	R	I	R	R	R	S	I	I	R	R
	B8-3 : *Escherichia coli* (99.9)	R	I	R	R	R	S	I	I	R	R

R: resistant. I: intermediate resistance. S: susceptible. Score in %: the similarity of strains by the analysis of 16S rRNA sequence using BLAST analysis.

B. Resistance to multiple antibiotics

We tested the susceptibility of bacterial isolates to various combinations of commonly used antibiotics. The MAR frequency of the bacterial isolates among the samples is shown in Table 2. All of the samples showed a broad range of MAR except the Sangweol river sample (Water-SR). A high MAR frequency, up to 0.37 %, was found in the fecal-contaminated water sample (Water-PF) collected from the pig farm (Table 2). In addition, the resistant isolates displayed an approximately 5-fold increase in the incidences of MAR to combinations of two different antibiotics (~3.1%) compared to combinations of three (~1.31%) or more (~0.56%) antibiotics. We also carried out an antibacterial susceptibility test with the disc diffusion assay for ten kinds of antibiotics used commonly in farm animals. The MAR patterns of bacterial isolates among samples are shown in Table 3. Most of the resistant isolates showed MAR patterns for 7 or more antibiotics, and the resistance patterns were similar among the sampling groups. Notably, the animal fecal-contaminated water samples (Water-CF and -PF) showed a wide range of multiple resistant patterns in the ten combinatorial antibiotic mixing sets tested (Table 3). To examine the MIC of the antibiotics for resistant isolates, we next tested the MIC with CEF, TET, GEN, ERY, and VAN. The MIC values for selected resistant isolates are shown in Table 4. Overall, higher CEF MICs were detected among selected resistant isolates (Table IV).

TABLE IV
MIC DATA FOR CEFAZOLIN, TETRACYCLINE, GENTAMYCIN, ERYTHROMYCIN, AND VANCOMYCIN AMONG ISOLATED BACTERIA FROM DIFFERENT SAMPLES

Samples	Strain code	CEF		TET		GEN		ERY		VAN	
		$MIC_{(\mu g/ml)}$	MIC_{range}	$MIC_{(\mu g/ml)}$	MIC_{range}	$MIC_{(\mu g/ml)}$	MIC_{range}	$MIC_{(\mu g/ml)}$	MIC_{range}	$MIC_{(\mu g/ml)}$	MIC_{range}
Water-SR	B1-1	≤ 1500	300 - 1500	≤ 100	10 - 100	≤ 10	1 - 500	> 750	> 750	≤ 60	6 - 300
Water-GR	B2-1	≤ 1500	300 - 1500	≤ 1	0.5 - 100	≤ 500	100 - 500	≤ 150	15 - 750	≤ 300	60 - 300
Water-CF	B3-1	≤ 1500	300 - 1500	< 0.5	< 0.5	≤ 100	10 - 500	≤ 150	15 - 750	≤ 60	6 - 300
Water-PF	B4-1	> 1500	> 1500	≤ 1	0.5 - 100	≤ 100	10 - 500	≤ 750	150 - 750	> 300	> 300
Water-SM	B5-1	≤ 1500	300 - 1500	≤ 100	10 - 100	≤ 1	0.5 - 500	≤ 750	150 - 750	≤ 60	6 - 300
Water-FM	B6-1	≤ 1500	30 - 1500	≤ 1	≤ 1 - 100	< 0.5	< 0.5	> 750	> 750	≤ 60	6 - 300
Meat-C	B7-1	≤ 1500	300 - 1500	≤ 100	10 - 100	≤ 1	0.5 - 500	≤ 750	150 - 750	≤ 60	6 - 300
Meat-P	B8-1	≤ 300	30 - 1500	≤ 100	10 - 100	≤ 10	1 - 500	≤ 750	150 - 750	≤ 60	6 - 300

C. Bacterial identification

We examined the bacterial species composition of the resistant isolates by 16S rRNA sequence analysis. Despite the fact that many colonies were initially selected for the isolation of resistant bacteria, only three isolates per group were further analyzed. The results of the 16S rRNA sequence analysis are shown in Table 3. These isolates were identified and classified into 7 different genera (Table 3). Among the identified resistant bacteria, *Pseudomonas* and *Escherichia* were the most abundant, followed by *Delftia* and *Enterobacter*; the other bacterial genera occupied a small fraction of the total selected isolates. The genera *Escherichia* and *Enterobacter* were major in the pig and chicken farm and meat samples, whereas *Delftia* was mainly detected in the river samples. Animal fecal samples showed a variety of bacterial genera, indicating that the animal fecal-contaminated waters derived from farm animals had highly diverse microbial communities. In addition, the genera *Delftia* and *Enterobacter* from the chicken farm showed a wide range of resistances for all of antibiotics tested (Table 3). The genera *Escherichia* and *Pseudomonas* isolated showed a variety of AR patterns to the ten antibiotics tested (Table 3). Finally, we carried out the phylogenetic analysis with isolated resistant bacteria as shown in Fig. 2. Most of the resistant isolates appeared to be dominated by the classes *Betaproteobacteria and Gammaproteobacteria* in the phylum *Proteobacteria* (Fig. 2).

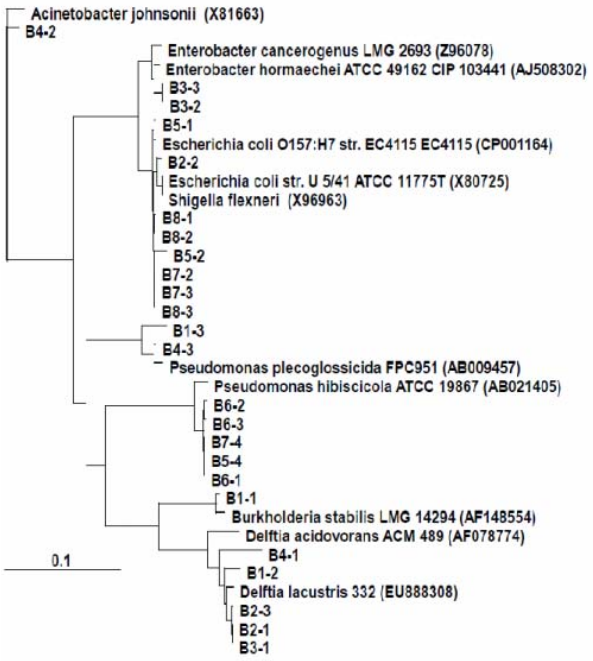

Fig. 2 Phylogenetic tree of resistant isolates based on almost-complete 16S rRNA sequence. The resultant 16S rRNA sequences were aligned together with corresponding sequences from representative species using the PHYDIT program. Scale bar, 10% nucleotide sequence divergence

IV. Discussion

AR is one of the major public health threats of the 21st century. The number of multidrug-resistant bacteria is rising in both clinical and community settings, and serious infections caused by resistant bacteria have become an emerging worldwide problem [1,2]. The increase of antibiotic usage in the animal farming industry is thought to be one of the major sources contributing to the emergence of AR, MAR or superbugs [1,2,3,4]. From an ecological point of view concerning current problems of AR, in this study, we addressed AR profiles and the correlation between animal-borne bacterial contamination and the prevalence of AR or MAR from animal farming aquatic environments and meats.

Since South Korea uses approximately 1.5 times more antibiotics than do other OECD countries, the recent emergence rates of antimicrobial and multidrug resistances were significantly higher than in past years or in other countries [16,17,18,19]. In this study, the animal fecal samples showed a significantly higher prevalence and a wider range of resistances to commonly used antibiotics in animal farms in Korea. Similar to previous reports [18,19,20], our results showed a high incidence of tetracycline resistance (~ 26 %) in animal-borne bacteria samples collected from animal fecal samples and water samples near animal farms. Tetracycline is one of the widely used antibiotics in veterinary medicine for the treatment of infections or as growth promoters on animal farms in South Korea, and it may explain the finding that tetracycline-resistant *Enterobacteriaceae* were routinely isolated from all farm animals [18,19,20]. Although the emergence of resistance led to its declining medicinal usage, tetracyclines still remain the first‐line medicine for a variety of applications, including acne vulgaris, cholera, Lyme disease, and pneumonia [1,2,20]. In addition, we found that the animal fecal samples showed a relatively higher resistance value (~ 20 %) for three antibiotics. These results also coincide with previous reports [21,22,23]. Vancomycin resistance is especially widespread among isolates from farm animals, which may serve as a reservoir for vancomycin-resistant enterococci. These bacteria may enter the human food chain and are one potential source of superbug emergence [21,22,24,25,26,27].

Despite the fact that antibiotics act as an important ecological factor in the aquatic environment that could potentially affect microbial communities, the effects of antibiotics in the aquatic environment are less studied than in the soil environment [5]. Large amounts of overused antibiotics are excreted by farm animals, and these livestock wastes can thus pollute aquatic environments, leading to the development of antibiotic-resistant populations in aquatic microbial communities [1,2,5]. This is supported by several studies that suggest that the increase of these resistant bacteria may be promoted in animal fecal-contaminated aquatic environments with high levels of antibiotics [28,29,30,31,32]. In this study, we showed that all of the resistant isolates selected belonged to the phylum *Proteobacteria*, according to the phylogenetic analysis of the 16S rRNA sequence. In the phylum *Proteobacteria*, the resistant isolates appeared to be dominated

by the classes *Betaproteobacteria and Gammaproteobacteria*, including the genera *Delftia*, *Burkholderia*, *Escherichia*, *Enterobacter*, *Acinetobacter*, *Shigella* and *Pseudomonas*. Although these genera are already known bacteria for MAR [1,2,3,4], our data indicate that resistance to antibiotics was more pronounced near farms than samples collected away from farms; therefore, the current resistance can be directly attributed to the broad usage of antibiotics in animal farms and not to an already underlying resistance in these species. Therefore, considering these facts, science-based regular monitoring of multi-antibiotic resistant bacteria derived from animal farms or near habitats has become an important process for the animal farming industry and public health.

ACKNOWLEDGMENT

This work was supported by a Youth Science Camp Grant from the Korea Foundation for the Advancement of Science and Creativity (2010-2011), the National Research Foundation of Korea Grant funded by the Korean Government (NRF-2009-0072758) and the Ministry of National Defense Foundation Grant (ADD: 08-10-02).

REFERENCES

[1] Alanis, A.J. 2005. Resistance to antibiotics: are we in the post-antibiotic era? *Arch Med Res* 36:697-705.
[2] Levy, S.B., and Marshall, B. 2004. Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med* 10:S122-129.
[3] Mathew, A.G., Cissell, R., and Liamthong, S. 2007. Antibiotic resistance in bacteria associated with food animals: a United States perspective of livestock production. *Foodborne Pathog Dis* 4:115-133.
[4] Shryock, T.R., and Richwine, A. 2010. The interface between veterinary and human antibiotic use. *Ann N Y Acad Sci* 1213:92-105.
[5] Ding, C., and He, J. 2010. Effect of antibiotics in the environment on microbial populations. *Appl Microbiol Biotechnol* 87:925-941.
[6] Davies, J. 1994. Inactivation of antibiotics and the dissemination of resistance genes. *Science* 264:375-382.
[7] Davison, J. 1999. Genetic exchange between bacteria in the environment. *Plasmid* 42:73-91.
[8] Alekshun, M.N., and Levy, S.B. 2007. Molecular mechanisms of antibacterial multidrug resistance. *Cell* 128:1037-1050.
[9] Kimiran-Erdem, A., Arslan, E.O., Sanli Yurudu, N.O., Zeybek, Z., Dogruoz, N., and Cotuk, A. 2007. Isolation and identification of enterococci from seawater samples: assessment of their resistance to antibiotics and heavy metals. *Environ Monit Assess* 125:219-228.
[10] Servais, P., and Passerat, J. 2009. Antimicrobial resistance of fecal bacteria in waters of the Seine river watershed (France). *Sci Total Environ* 408:365-372.
[11] Witte, W. 2000. Ecological impact of antibiotic use in animals on different complex microflora: environment. *Int J Antimicrob Agents* 14:321-325.
[12] Kilonzo-Nthenge, A., Nahashon, S.N., Chen, F., and Adefope, N. 2008. Prevalence and antimicrobial resistance of pathogenic bacteria in chicken and guinea fowl. *Poult Sci* 87:1841-1848.
[13] Bauer, A.W., Kirby, W.M., Sherris, J.C., and Turck, M. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45:493-496.
[14] Reboucas, R.H., de Sousa, O.V., Lima, A.S., Vasconcelos, F.R., de Carvalho, P.B., and Vieira, R.H. 2011. Antimicrobial resistance profile of Vibrio species isolated from marine shrimp farming environments (Litopenaeus vannamei) at Ceara, Brazil. *Environ Res* 111:21-24.
[15] Cho, S.H., Han, J.H., Ko, H.Y., and Kim, S.B. 2008. Streptacidiphilus anmyonensis sp. nov., Streptacidiphilus rugosus sp. nov. and Streptacidiphilus melanogenes sp. nov., acidophilic actinobacteria isolated from Pinus soils. *Int J Syst Evol Microbiol* 58:1566-1570.
[16] Hur, J., Choi, Y.Y., Park, J.H., Jeon, B.W., Lee, H.S., Kim, A.R., and Lee, J.H. 2011. Antimicrobial resistance, virulence-associated genes, and pulsed-field gel electrophoresis profiles of Salmonella enterica subsp. enterica serovar Typhimurium isolated from piglets with diarrhea in Korea. *Can J Vet Res* 75:49-56.
[17] Kang, M.S., Kim, A., Jung, B.Y., Her, M., Jeong, W., Cho, Y.M., Oh, J.Y., Lee, Y.J., Kwon, J.H., and Kwon, Y.K. 2010. Characterization of antimicrobial resistance of recent Salmonella enterica serovar Gallinarum isolates from chickens in South Korea. *Avian Pathol* 39:201-205.
[18] Nam, H.M., Lim, S.K., Moon, J.S., Kang, H.M., Kim, J.M., Jang, K.C., Kang, M.I., Joo, Y.S., and Jung, S.C. 2010. Antimicrobial resistance of enterococci isolated from mastitic bovine milk samples in Korea. *Zoonoses Public Health* 57:e59-64.
[19] Oh, E.G., Son, K.T., Yu, H., Lee, T.S., Lee, H.J., Shin, S., Kwon, J.Y., Park, K., and Kim, J. 2011. Antimicrobial resistance of Vibrio parahaemolyticus and Vibrio alginolyticus strains isolated from farmed fish in Korea from 2005 through 2007. *J Food Prot* 74:380-386.
[20] Unno, T., Han, D., Jang, J., Lee, S.N., Kim, J.H., Ko, G., Kim, B.G., Ahn, J.H., Kanaly, R.A., Sadowsky, M.J., et al. 2010. High diversity and abundance of antibiotic-resistant Escherichia coli isolated from humans and farm animal hosts in Jeonnam Province, South Korea. *Sci Total Environ* 408:3499-3506.
[21] Lim, S.K., Lee, H.S., Nam, H.M., Cho, Y.S., Kim, J.M., Song, S.W., Park, Y.H., and Jung, S.C. 2007. Antimicrobial resistance observed in Escherichia coli strains isolated from fecal samples of cattle and pigs in Korea during 2003-2004. *Int J Food Microbiol* 116:283-286.
[22] Li, D., Yu, T., Zhang, Y., Yang, M., Li, Z., Liu, M., and Qi, R. 2010. Antibiotic resistance characteristics of environmental bacteria from an oxytetracycline production wastewater treatment plant and the receiving river. *Appl Environ Microbiol* 76:3444-3451.
[23] Joseph, S.W., Hayes, J.R., English, L.L., Carr, L.E., and Wagner, D.D. 2001. Implications of multiple antimicrobial-resistant enterococci associated with the poultry environment. *Food Addit Contam* 18:1118-1123.
[24] Hammerum, A.M., Lester, C.H., and Heuer, O.E. 2010. Antimicrobial-resistant enterococci in animals and meat: a human health hazard? *Foodborne Pathog Dis* 7:1137-1146.
[25] Heuer, O.E., Hammerum, A.M., Collignon, P., and Wegener, H.C. 2006. Human health hazard from antimicrobial-resistant enterococci in animals and food. *Clin Infect Dis* 43:911-916.
[26] Butaye, P., Devriese, L.A., Goossens, H., Ieven, M., and Haesebrouck, F. 1999. Enterococci with acquired vancomycin resistance in pigs and chickens of different age groups. *Antimicrob Agents Chemother* 43:365-366.
[27] Butaye, P., Devriese, L.A., and Haesebrouck, F. 1999. Comparison of direct and enrichment methods for the selective isolation of vancomycin-resistant enterococci from feces of pigs and poultry. *Microb Drug Resist* 5:131-134.
[28] Bates, J. 1997. Epidemiology of vancomycin-resistant enterococci in the community and the relevance of farm animals to human infection. *J Hosp Infect* 37:89-101.
[29] Bates, J., Jordens, J.Z., and Griffiths, D.T. 1994. Farm animals as a putative reservoir for vancomycin-resistant enterococcal infection in man. *J Antimicrob Chemother* 34:507-514.
[30] Knapp, C.W., Engemann, C.A., Hanson, M.L., Keen, P.L., Hall, K.J., and Graham, D.W. 2008. Indirect evidence of transposon-mediated selection of antibiotic resistance genes in aquatic systems at low-level oxytetracycline exposures. *Environ Sci Technol* 42:5348-5353.
[31] Fan, C., Lee, P.K., Ng, W.J., Alvarez-Cohen, L., Brodie, E.L., Andersen, G.L., and He, J. 2009. Influence of trace erythromycin and eryhthromycin-H2O on carbon and nutrients removal and on resistance selection in sequencing batch reactors (SBRs). *Appl Microbiol Biotechnol* 85:185-195.
[32] Esiobu, N., Armenta, L., and Ike, J. 2002. Antibiotic resistance in soil and water environments. *Int J Environ Health Res* 12:133-144.
[33] Biyela, P.T., Lin, J., and Bezuidenhout, C.C. 2004. The role of aquatic ecosystems as reservoirs of antibiotic resistant bacteria and antibiotic resistance genes. *Water Sci Technol* 50:45-50.
[34] Schwartz, T., Kohnen, W., Jansen, B., and Obst, U. 2003. Detection of antibiotic-resistant bacteria and their resistance genes in wastewater, surface water, and drinking water biofilms. *FEMS Microbiol Ecol* 43:325-335.