ORIGINAL ARTICLE

J Bacteriol Virol. Vol 53. No 2. December 2023; 53(2): 141–149
https://doi.org/10.4167/jbv.2023.53.2.141
pISSN 1598–2467 / eISSN 2093–0429

JBV

Reduction in Antimicrobial Resistance Prevalence in *Escherichia coli* and *Enterococcus* Species from a Swine Farm in Korea following All–In/All–Out Management

Chang Min Jung[1,2], Kyung-Hyo Do[2], Kwangwon Seo[2*]

[1]Onnuri Animals Hospital, Munamgil 16, Dongnam-Gu, Choen-an 31070, Republic of Korea
[2]College of Veterinary Medicine, Chungbuk National University, Cheongju 28644, Republic of Korea

Corresponding
Kwangwon Seo, DVM, PhD, Professor
College of Veterinary Medicine,
Chungbuk National University,
Cheongju 28644, Republic of Korea
Phone : +82–43–261–2907
E–mail : vetskw16@cbnu.ac.kr

Received : December 3, 2023
Revised : December 17, 2023
Accepted : December 20, 2023

No potential conflict of interest relevant to this article was reported.

All-In/All-Out (AIAO) is a management strategy in pig farming where all animals within a specific production group are introduced into a facility at the same time and are subsequently removed together when the production cycle is complete. The aim of this study was to analyzing whether the application of AIAO system in pig farm in Korea affects the reduction of antimicrobial resistance. A total of 60 *Escherichia coli* (*E. coli*) and 60 *Enterococcus* species (spp.) isolates were tested in this study: 30 from "Before application of AIAO", and "After application of AIAO". All *E. coli* and *Enterococcus* spp. isolates were tested for antimicrobial susceptibility using the disc diffusion test. We found that there were significant decrease of resistance rates to cefoxitin (40.0% to 20.0%), cefepime (20.0% to 0.0%), gentamicin (50.0% to 20.0%), kanamycin (80.0% to 40.0%), sulfisoxazole (73.3% to 40.0%), and trimethoprim-sulfamethoxazole (86.7% to 40.0%) in *E. coli*, and ampicillin (6.7% to 0.0%), penicillin (53.3% to 40.0%), ciprofloxacin (66.7% to 40.0%), and gentamicin (86.7% to 60.0%) in *Enterococcus* spp. after the application of AIAO. Through this, we suggest the application of AIAO management system as the decreasing antimicrobial resistance model for pig farms. This could contribute to the development of approaches for research on antimicrobial resistance and its effective management.

Key Words: Swine, Antimicrobial resistance, All-In/All-Out, *Escherichia coli*, *Enterococcus* spp.

INTRODUCTION

The utilization of antimicrobial agents stands out as a highly economical approach to maintain or improve the health and feed efficiency of animals raised in the context of traditional agricultural methods (1). In intensive animal production, particularly within the pig production sector, antimicrobial usage rates rank among the highest, considering both absolute values and treatment incidence (2).

Excessive and improper utilization of antimicrobials in veterinary medicine has resulted in the rise of antimicrobial resistant bacteria (1). In light of the risk posed by the prevalence of infectious diseases stemming from non-treatable, multi-drug resistant bacteria to antimicrobials, considerable endeavors need to be undertaken to curb their emergence and dissemination (3). Hence, there is a necessity

to enhance the management of bacterial infections through methods alternative to the use of antimicrobial agents.

All-In/All-Out (AIAO) is a management strategy in pig farming where all animals within a specific production group are introduced into a facility at the same time and are subsequently removed together when the production cycle is complete (4). Production systems employing AIAO practices enhance both growth performance and feed efficiency while concurrently mitigating the risk of disease transmission. Pigs are collectively moved to fresh housing as they progress through the production stages, ensuring adequate intervals between batches for thorough cleaning, disinfection, and drying of the facilities (4). AIAO enhances biosecurity practices by providing clear periods when facilities can be thoroughly cleaned and disinfected. This is crucial for preventing the introduction and transmission of pathogens that may contribute to the need for antimicrobial treatments (5).

AIAO reduces the mixing of animals from different groups, there by restricting interactions that could lead to the exchange of bacteria carrying antimicrobial resistance genes (6). This helps prevent the spread of resistant strains within the farm environment (5, 6). Also, AIAO contributes to reducing the overall environmental impact of use of antimicrobial agents (4-6). Effective disease control and management can reduce the frequency of antibiotic use, thereby lowing the environmental risk of antibiotic residues, which can contribute to reducing the risk of antibiotic resistance in environmental bacteria (5-7). The aim of this study was to analyzing whether the application of AIAO system in pig farm in Korea affects the reduction of antimicrobial resistance.

Materials and Methods

Experimental Design

To analyzing reduction in antimicrobial resistance through the application of the AIAO system, a total of five farms were recruited to apply the AIAO system, which sufficiently disinfects/dried the pig house before the next pig entry. All experimental farms housed about 500 sows, and AIAO system were applied at pigsty for weaned piglets (21 days-old to 70 days-old piglets).

Samples were collected from March 2022 to July 2022. Before the application of AIAO system, feces and dust samples were collected from pig farms. Samples were collected only once at 7 days before the pigs were out from the pigsty (March 2022). Also, three months after the application of AIAO system, feces and dust samples were collected from same space of pig farms, as the same manners (July 2022)

To gather feces and dust samples, a sterile surgical gauze swab was dampened using 10 mL of sterile phosphate-buffered saline solution. About 10 g of fecal samples were collected, and two distinct sections of pigsties were swabbed to obtain approximately 10 g of dust samples. All samples were transported aseptically to the laboratory with 4℃ for isolating *Escherichia coli* (*E. coli*) and *Enterococcus* species. All collected samples were transported to laboratory with ice-pack on the day of collection.

Isolation of E. coli

Aseptically collected feces and dust samples were individually inoculated into 5 mL of Modified Esculin (mEC; Becton-Dickinson, MD, USA) broth media at 37℃ for 24 h. After incubation, mEC media was streaked onto MacConkey (Becton-Dickinson) agar media and incubated at 37℃for 24 h. Typical pink colored-colonies were selected from each sample. Identification of *E. coli* was confirmed using polymerase chain reaction according to previously described study (8). Briefly, each selected colonies were heated at 95℃ for 5 min. The centrifuged supernatant was used as a DNA template. For identification of *E. coli*, 4 μM of forward primer (malB-F: 5'-GACCTCGGTTTAGTTCACAGA-3') and reverse primer

(malB-R: 5'-CACACGCTGACGCTGACCA-3') were used. After amplification, the products were visualized by electrophoresis in 1.5% agarose gels stained with HiQ BlueMango (bioD, Seoul, Korea) under UV light, and products that showed size of 585 bp were identified as *E. coli*. All *E. coli* isolates were evenly isolated from each experimental farm (6 isolates before the application of AIAO, and 6 isolates after the application of AIAO from each of farms). A total of 60 *E. coli* isolates were tested in this study: 30 from "Before application of AIAO", and "After application of AIAO".

Isolation of Enterococcus species

Aseptically collected feces and dust samples were individually inoculated into 5 mL of Enterococcosel (Becton-Dickinson) broth media at 37℃ for 24 h. After incubation, Enterococcosel broth media was streaked onto Enterococcosel (Becton-Dickinson) agar media and incubated at 37℃ for 24 h. Typical black colored-colonies were selected from each sample. Identification of *Enterococcus* species was confirmed using polymerase chain reaction according to previously described study (8). Briefly, each selected colonies were heated at 95℃ for 5 min. The centrifuged supernatant was used as a DNA template. For identification of *Enterococcus faecalis*, 4 μM of forward primer (ddl-1-F: 5'-TGTTGTATGGCG GCAGAAGT -3') and reverse primer (ddl-1-R: 5'-TCAGGTGTTTGTGCCCAAGT-3') were used. *Enterococcus faecium* was identified using 4 μM of forward primer (ddl-2-F: 5'-ATGGACCCAAGTGGACAGA -3') and reverse primer (ddl-2-R: 5'-ATTTCGCGCGCTTCAATTCC-3'). After amplification, the products were visualized by electrophoresis in 2.0% agarose gels stained with HiQ BlueMango (bioD, Seoul, Korea) under UV light, and products that showed size of 199 bp were identified as *E. faecalis*, and size of 474 bp were identified as *E. faecium*. All *Enterococcus* spp. isolates were evenly isolated from each experimental farm (6 isolates before the application of AIAO, and 6 isolates after the application of AIAO from each of farms). A total of 60 *Enterococcus* spp. isolates were tested in this study: 30 from "Before application of AIAO", and "After application of AIAO".

Antimicrobial Susceptibility Test

All *E. coli* and *Enterococcus* spp. isolates were tested for antimicrobial susceptibility using the disc diffusion test. The following antimicrobial agents were selected for testing *E. coli* isolates after referring to the Clinical and Laboratory Standards Institute (CLSI) guidance (9): ampicillin (10 μg), amoxicillin-clavulanic acid (20/10 μg), cefazolin (30 μg), cefoxitin (30 μg), ceftiofur (30 μg), ceftazidime (30 μg), cefepime (30 μg), gentamicin (10 μg), streptomycin (10 μg), kanamycin (30 μg), oxytetracycline (30 μg), tetracycline (30 μg), florfenicol (30 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), sulfisoxazole (250 μg), trimethoprim-sulfamethoxazole (1.25/23.75 μg), and colistin (10 μg). Also, for testing antimicrobial susceptibility of *Enterococcus* spp. following antimicrobial agents were used: ampicillin (10 μg), penicillin (10 U), tylosin (30 μg), erythromycin (15 μg), doxycycline (30 μg), tetracycline (30 μg), tigecycline (15 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), vancomycin (30 μg), florfenicol (30 μg), chloramphenicol (30 μg), gentamicin (10 μg), kanamycin (30 μg), and streptomycin (10 μg).

The acquisition of all antimicrobial discs was made from Becton-Dickinson. Isolates exhibiting resistance to three or more CLSI subclasses were classified as multi-drug resistant strains (10).

Statistical analysis

The statistical analysis was conducted utilizing the SPSS version 12.0 software (SPSS, Chicago, Illinois, USA). The analysis of antimicrobial resistance rates of *E. coli* and *Enterococcus* spp. from pig farms was conducted using the Chi-square test.

Results

Antimicrobial resistance and Multi-drug resistance of E. coli

The antimicrobial resistance of *E. coli* isolated from pig farms were described in Table 1. The resistance rates to ampicillin, cefazolin, ceftiofur, streptomycin, oxytetracycline, tetracycline, florfenicol, chloramphenicol, nalidixic acid, and ciprofloxacin were more than 50.0%. We found that the resistance rates to cefazolin, cefoxitin, ceftazidime, cefepime, gentamicin,

Table 1. Antimicrobial resistance phenotype of *E. coli* isolated from pig farms before and after all-in/all-out management

Antimicrobial subclasses Antimicrobial agents	No. of resistant isolates (Antimicrobial resistance %)*	
	Before all-in/all-out management (n=30)	After all-in/all-out management (n=30)
Aminopenicillins		
Ampicillin	26 (86.7%)	27 (90.0%)
β-lactam / β-lactamase inhibitor combinations		
Amoxicillin-clavulanic acid	12 (40.0%)	12 (40.0%)
1st generation of cephalosporins		
Cefazolin	22 (73.3%)	21 (70.0%)
2nd generation of cephalosporins		
Cefoxitin	12 (40.0%)[a]	6 (20.0%)[b]
3rd generation of cephalosporins		
Ceftiofur	30 (100.0%)	30 (100.0%)
Ceftazidime	6 (20.0%)	3 (10.0%)
4th generation of cephalosporins		
Cefepime	6 (20.0%)[a]	0 (0.0%)[b]
Aminoglycosides		
Gentamicin	15 (50.0%)[a]	6 (20.0%)[b]
Streptomycin	24 (80.0%)[b]	27 (90.0%)[a]
Kanamycin	24 (80.0%)[a]	12 (40.0%)[b]
1st generation of tetracyclines		
Tetracycline	26 (86.7%)[b]	30 (100.0%)[a]
Oxytetracycline	25 (83.3%)[b]	30 (100.0%)[a]
Phenicol		
Florfenicol	22 (73.3%)[b]	27 (90.0%)[a]
Chloramphenicol	27 (90.0%)[b]	30 (100.0%)[a]
Quinolones		
Nalidixic acid	24 (80.0%)	24 (80.0%)
Fluoroquinolones		
Ciprofloxacin	23 (76.7%)	21 (70.0%)
Sulfonamides		
Sulfisoxazole	22 (73.3%)[a]	12 (40.0%)[b]
Trimethoprim-sulfamethoxazole	26 (86.7%)[a]	12 (40.0%)[b]
Lipopeptides		
Colistin	1 (3.3%)	3 (10.0%)

*Different superscript letters (a and b) means statistically different group by chi-square test ($p < 0.05$).

Table 2. Multi-drug resistance of *E. coli* isolated from pig farms before and after all-in/all-out management

No. of resistance	No. of resistant isolates (Antimicrobial resistance %)*	
	Before all-in/all-out management (n=30)	After all-in/all-out management (n=30)
0 subclass	0 (0.0%)	0 (0.0%)
1 subclass	0 (0.0%)	0 (0.0%)
2 subclasses	0 (0.0%)	0 (0.0%)
3 subclasses	1 (3.3%)	1 (3.3%)
4 subclasses	2 (6.7%)[a]	0 (0.0%)[b]
5 subclasses	5 (16.7%)	5 (16.7%)
6 subclasses	4 (13.3%)[b]	6 (20.0%)[a]
7 subclasses	2 (6.7%)[b]	4 (13.3%)[a]
8 subclasses	5 (16.7%)	5 (16.7%)
9 subclasses	11 (36.7%)	9 (30.0%)
Multi-Drug Resistance ($\geq$ 3 subclasses)	30 (100.0%)	30 (100.0%)

*Different superscript letters (a and b) means statistically different group by chi-square test ($p < 0.05$).

kanamycin, ciprofloxacin, sulfisoxazole, and trimethoprim-sulfamethoxazole were lower in *E. coli* isolates after the application of AIAO. Moreover, after the application of AIAO, there were significant decrease of resistance rates to cefoxitin (40.0% to 20.0%), cefepime (20.0% to 0.0%), gentamicin (50.0% to 20.0%), kanamycin (80.0% to 40.0%), sulfisoxazole (73.3% to 40.0%), and trimethoprim-sulfamethoxazole (86.7% to 40.0%). There was no difference of multi-drug resistance ratio between before and after the application of AIAO (Table 2) however, resistant to 9 antimicrobial subclasses were decreased (36.7% to 30.0%) after the application of AIAO.

Antimicrobial resistance and Multi-drug resistance of Enterococcus spp.

Table 3 described the antimicrobial resistance of *Enterococcus* spp. isolated from pig farms. The resistance rates to tylosin, erythromycin, doxycycline, tetracycline, nalidixic acid, florfenicol, chloramphenicol, gentamicin, kanamycin, and streptomycin were more than 50.0%. After the application of AIAO, the antimicrobial resistance rates to ampicillin, penicillin, erythromycin, doxycycline, tetracycline, ciprofloxacin, florfenicol, and gentamicin were decreased. Also, we found that resistance rates to ampicillin (6.7% to 0.0%), penicillin (53.3% to 40.0%), ciprofloxacin (66.7% to 40.0%), and gentamicin (86.7% to 60.0%) were significantly decreased after the application of AIAO. Like the results of *E. coli*, there was no difference of multi-drug resistance ratio between before and after the application of AIAO (Table 4). However, resistant to 9 antimicrobial subclasses were significantly decreased (10.0% to 0.0%) after the application of AIAO.

Discussion

In this study, we analyzed the antimicrobial resistance of *E. coli* and *Enterococcus* spp. isolated from 5 pig farms before and after the application of AIAO management system. For the analyzing the antimicrobial resistance of pig farms, we selected *E. coli* and *Enterococcus* spp. because those are intestinal commensal bacteria present in swine, and many countries' antimicrobial surveillance systems were widely used *E. coli* and *Enterococcus* spp. as good indicators for monitoring the general level of antimicrobial resistance (1, 11, 12).

Table 3. Antimicrobial resistance phenotype of *Enterococcus* species isolated from pig farms before and after all-in/all-out management

Antimicrobial subclasses Antimicrobial agents	No. of resistant isolates (Antimicrobial resistance %)*	
	Before all-in/all-out management (n=30)	After all-in/all-out management (n=30)
Aminopenicillins		
Ampicillin	2 (6.7%)[a]	0 (0.0%)[b]
Penicillins		
Penicillin	16 (53.3%)[a]	12 (40.0%)[b]
Macrolides		
Tylosin	28 (93.3%)[b]	30 (100.0%)[a]
Erythromycin	28 (93.3%)	27 (90.0%)
1st generation of tetracyclines		
Tetracycline	28 (93.3%)	27 (90.0%)
2nd generation of tetracyclines		
Doxycycline	20 (66.7%)	15 (50.0%)
3rd generation of tetracyclines		
Tigecycline	0 (0.0%)	0 (0.0%)
Quinolones		
Nalidixic acid	26 (86.7%)[b]	30 (100.0%)[a]
Fluoroquinolones		
Ciprofloxacin	20 (66.7%)[a]	12 (40.0%)[b]
Glycopeptides		
Vancomycin	0 (0.0%)	0 (0.0%)
Phenicols		
Florfenicol	22 (73.3%)	18 (60.0%)
Chloramphenicol	22 (73.3%)[b]	27 (90.0%)[a]
Aminoglycosides		
Gentamicin	26 (86.7%)[a]	18 (60.0%)[b]
Kanamycin	26 (86.7%)[b]	30 (100.0%)[a]
Streptomycin	28 (93.3%)[b]	30 (100.0%)[a]

*Different superscript letters (a and b) means statistically different group by chi-square test ($p < 0.05$).

The effect on reducing antimicrobial resistance of AIAO management system was investigated in 5 pig farms. We found that when pig farms applicated AIAO management system, the antimicrobial resistance was significantly decreased. We found that there were significant decrease of resistance rates to cefoxitin (40.0% to 20.0%), cefepime (20.0% to 0.0%), gentamicin (50.0% to 20.0%), kanamycin (80.0% to 40.0%), sulfisoxazole (73.3% to 40.0%), and trimethoprim-sulfamethoxazole (86.7% to 40.0%) in *E. coli*, and ampicillin (6.7% to 0.0%), penicillin (53.3% to 40.0%), ciprofloxacin (66.7% to 40.0%), and gentamicin (86.7% to 60.0%) in *Enterococcus* spp. after the application of AIAO. In AIAO system, Pigs are collectively moved to fresh housing as they progress through the production stages, ensuring adequate intervals between batches for thorough cleaning, disinfection, and drying of the facilities (4). One of the important reasons for high antimicrobial resistance is the spread of resistance due to environmentally residual antimicrobial resistant bacteria, and it is thought that resistance had decreased due to the application of the AIAO management system that complies with clean and disinfect (7, 13, 14).

Table 4. Multi-drug resistance of *Enterococcus* species isolated from pig farms before and after all-in/all-out management

No. of resistance	No. of resistant isolates (Antimicrobial resistance %)*	
	Before all-in/all-out management (n=30)	After all-in/all-out management (n=30)
0 subclass	0 (0.0%)	0 (0.0%)
1 subclass	0 (0.0%)	0 (0.0%)
2 subclasses	1 (3.3%)	1 (3.3%)
3 subclasses	2 (6.7%)	2 (6.7%)
4 subclasses	4 (13.3%)	3 (10.0%)
5 subclasses	2 (6.7%)	3 (10.0%)
6 subclasses	4 (13.3%)[b]	7 (23.3%)[a]
7 subclasses	6 (20.0%)	6 (20.0%)
8 subclasses	8 (26.7%)	8 (26.7%)
9 subclasses	3 (10.0%)[a]	0 (0.0%)[b]
Multi-Drug Resistance (≥ 3 subclasses)	29 (99.7%)	29 (99.7%)

*Different superscript letters (a and b) means statistically different group by chi-square test ($p < 0.05$).

The World Health Organization classifies antimicrobial agents into highest priority critically important antimicrobials (HPCIA), critical important antimicrobials (CIA), high import antimicrobials (HIA), and important antimicrobials (IA) groups to ensure antimicrobial susceptibility according to their medical importance in humans (3). Also, the World Organization for Animal Health also defines antimicrobial agents according to their importance as veterinary critically important antimicrobials (VCIA), veterinary highly important antimicrobials (VHIA), and veterinary important antimicrobials (VIA) (3). The cefepime and ciprofloxacin are antimicrobial agents classified as HPCIA and VCIA. The cefepime which is the 4th generation of cephalosporins, is commonly used in pig farms to control enteric diseases and are known for their extended duration of action, which allows for less frequent dosing (15). And ciprofloxacin which is the fluoroquinolones, is commonly used to treat severe infections such as typhoid, paratyphoid fever. Thus, the risk management strategies for these antimicrobial agents are urgently needed (16).

Also, we found that resistance rates to gentamicin and kanamycin were significantly decreased after the application of AIAO management system. Those are aminoglycosides antimicrobial agents which is classified as VCIA (17). Owing to the unfavorable outcomes linked to aminoglycosides, including inner ear toxicity leading to sensorineural hearing loss and kidney damage resulting in chronic kidney disease, the utilization of aminoglycosides is restricted to the treatment of severe infections in human patients (17). However, in the case of pig, aminoglycosides may be employed for the control of enteric disease (11). Administering aminoglycosides to pigs holds the risk of inducing cross-resistance to critically important human antimicrobials, such as amikacin, posing a significant threat to human health (1).

In this study, we found that resistance rates to cefepime, ciprofloxacin, gentamicin, and kanamycin were significantly decreased after the application of AIAO management system. Through this, we assumed that the application of AIAO management system as the decreasing antimicrobial resistance model for pig farms.

The limitation of this study includes relatively short study period. For a significant reduction in antimicrobial resistance, approximately 6 to 12 months are required (18). Therefore, it is presumed that observed multi-drug resistance rates showed no significant differences because of the relatively short study period. The reduction of multi-drug resistant bacteria in pigs is crucial for preventing the dissemination of antimicrobial resistance from livestock to humans (19).

Although there was no difference of multi-drug resistance between the application of AIAO management system, we found that there was significant decrease in *Enterococcus* spp. resistant to 9 antimicrobial subclasses, and significant increase in *Enterococcus* spp. resistant to 6 antimicrobial subclasses in this study. We presumed that increased rates of resistance patterns of 6 antimicrobial subclasses was caused because isolates resistant to 10 antimicrobial subclasses lost their resistance. *Enterococcus* spp. are known reservoirs of resistance genes, and their prevalence in pig populations raises concerns about the potential transfer of resistance to bacteria affecting human health (20). Addressing multi-drug resistance in pigs not only safeguards the efficacy of antimicrobial agents used in veterinary medicine but also plays a pivotal role in minimizing the risk of zoonotic transmission (5, 6). By mitigating the presence of resistant strains in pig farming, we contribute to the broader objective of preserving the effectiveness of antimicrobial treatments, promoting both animal welfare and public health (5).

Through this study, we confirmed that application of all-in/all-out management system lower antimicrobial resistance. This could contribute to the development of approaches for research on antimicrobial resistance and its effective management.

ACKNOWLEDGEMENTS

This work was supported by the National Research Foundation of Korea (NRF) grant funded by the Korea government (MSIT) (No. 2022R1F1A1076134).

REFERENCES

1) Do KH, Seo K, Lee WK. Antimicrobial resistance, virulence genes, and phylogenetic characteristics of pathogenic *Escherichia coli* isolated from patients and swine suffering from diarrhea. *BMC Microbiol* 2022;22:199.

2) Do KH, Byun JW, Lee WK. Antimicrobial Resistance, Adhesin and Toxin Genes of Porcine Pathogenic *Escherichia coli* Following the Ban on Antibiotics as the Growth Promoters in Feed. *Pak Vet J* 2021;41:519-23.

3) FAO. Joint FAO/WHO/OIE expert meeting on critically important antimicrobials. Rome: Electronic Publishing Policy and Support Branch Communication Division, 2008.

4) Rodrigues da Costa M, García Manzanilla E, Diana A, van Staaveren N, Torres-Pitarch A, Boyle LA, et al. Identifying challenges to manage body weight variation in pig farms implementing all-in-all-out management practices and their possible implications for animal health: a case study. *Porcine Health Manag* 2021;7:10.

5) Davies R, Wales A. Antimicrobial Resistance on Farms: A Review Including Biosecurity and the Potential Role of Disinfectants in Resistance Selection. *Compr Rev Food Sci Food Saf* 2019;18:753-74.

6) Yun J, Muurinen J, Nykäsenoja S, Seppä-Lassila L, Sali V, Suomi J, et al. Antimicrobial use, biosecurity, herd characteristics, and antimicrobial resistance in indicator *Escherichia coli* in ten Finnish pig farms. *Prev Vet Med* 2021;193:105408.

7) Van Gompel L, Luiken REC, Sarrazin S, Munk P, Knudsen BE, Hansen RB, et al. The antimicrobial resistome in relation to antimicrobial use and biosecurity in pig farming, a metagenome-wide association study in nine European countries. *J Antimicrob Chemother* 2019;74:865-76.

8) Candrian U, Furrer B, Höfelein C, Meyer R, Jermini M, Lüthy J. Detection of Escherichia coli and identification of enterotoxigenic strains by primer-directed enzymatic amplification of specific DNA sequences. Int J Food Microbiol 1991;12:339-51.

9) M100-S24: Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement. Wayne, PA, USA: CLSI, 2020.

10) Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin *Microbiol Infect* 2012;18:268-81.

11) Seo KW, Do KH, Lee WK. Molecular Characteristics of Fluoroquinolone-Resistant *Escherichia coli* Isolated From Suckling Piglets With Colibacillosis. *BMC Microbiol* 2022;22:216.

12) Agunos A, Gow SP, Léger DF, Carson CA, Deckert AE, Bosman AL, et al. Antimicrobial use and antimicrobial resistance indicators - integration of farm-level surveillance data from broiler chickens and turkeys in British Columbia, Canada. *Front Vet Sci* 2019;6:131.

13) Angulo FJ, Baker NL, Olsen SJ, Anderson A, Barrett TJ. Antimicrobial use in agriculture: Controlling the transfer of antimicrobial resistance to humans. *Semin Pediatr Infect Dis* 2004;15:78-85.

14) Pungpian C, Sinwat N, Angkititrakul S, Prathan R, Chuanchuen R. Presence and Transfer of Antimicrobial Resistance Determinants in Escherichia coli in Pigs, Pork, and Humans in Thailand and Lao PDR Border Provinces. *Microb Drug Resist* 2021;27:571-84.

15) Seo KW, Do KH, Jung CM, Lee SW, Lee YJ, Lim SK, et al. Comparative genetic characterization of third-generation cephalosporin-resistant *Escherichia coli* isolated from integrated and conventional pig farm in Korea. *J Glob Antimicrob Resist* 2023;34:74-82.

16) Huang K, Xu CW, Zeng B, Xia QQ, Zhang AY, Lei CW, et al. Dynamics of Quinolone Resistance in Fecal *Escherichia coli* of Finishing Pigs after Ciprofloxacin Administration. *J Vet Med Sci* 2014;76:1213-8.

17) Jackson CR, Fedorka-Cray PJ, Barrett JB, Ladely SR. High-level aminoglycoside resistant enterococci isolated from swine. *Epidemiol Infect* 2005;133:367-71.

18) Lee SW, Jung CM, Do KH, Lee WK, Seo KW. Effects of Antimicrobial Administration Route on Growth and Antimicrobial Resistance in Weaned Piglets. *Animals* 2023;13:3264.

19) Kennedy CA, Walsh C, Karczmarczyk M, O'Brien S, Akasheh N, Quirke M, et al. Multi-drug resistant *Escherichia coli* in diarrhoeagenic foals: Pulsotyping, phylotyping, serotyping, antibiotic resistance and virulence profiling. *Vet Microbiol* 2018;223:144-52.

20) Thu WP, Sinwat N, Bitrus AA, Angkittitrakul S, Prathan R, Chuanchuen R. Prevalence, antimicrobial resistance, virulence gene, and class 1 integrons of Enterococcus faecium and Enterococcus faecalis from pigs, pork and humans in Thai-Laos border provinces. *J Glob Antimicrob Resist* 2019;18:130-8.