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Effects of Liquid Administering Antimicrobial Agents on Reducing Antimicrobial Resistance in Weaned Piglets in Korea

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Antimicrobial agents are typically delivered through two primary oral routes: either liquid administering into drinking water or powder administering into feed. The aim of study was to analyze the effects of liquid administering antimicrobial agents on antimicrobial resistance. We selected 5 pig farms that have both a weaner house with a proportional liquid dispenser, and a weaner house that does not have proportional liquid dispenser, and confirmed that weaned piglets in weaner house with proportional liquid dispenser were treated antimicrobial agents via drinking water, and weaned piglets in weaner house without proportional liquid dispenser were treated antimicrobial agents with powder administering into feed. A total of 80 *Escherichia coli* (*E. coli*) and 79 *Enterococcus* species (spp.) isolates were tested in this study. All *E. coli* and *Enterococcus* spp. isolates were tested for antimicrobial susceptibility using the disc diffusion test. We found that isolates from house with proportional liquid feeding showed a significantly lower antimicrobial resistance rates to chloramphenicol (77.8%) in *E. coli* compared to that from house without proportional liquid dispenser (90.9%), and tetracycline (80.0%), florfenicol (77.5%), and kanamycin (87.5%) in *Enterococcus* spp. compared to those from house without proportional liquid dispenser (89.7%, 92.3%, and 97.4%, respectively). The findings suggest that utilizing drinking water as a means of administering antimicrobial agents is a favorable approach for disease management in pig production.

Key Words: Swine, Antimicrobial resistance, Liquid administering antimicrobial agents, *Escherichia coli*, *Enterococcus* spp.

INTRODUCTION

In conventional farming systems, piglets experience an abrupt weaning process at 3-4 weeks of age, which markedly differs from the more extended transition period observed in semi-natural rearing conditions, ranging from 15 to 22 weeks (1). Premature weaning can result in heightened stress levels, diminished feed intake, and suboptimal growth performance in piglets (2). Moreover, weaned piglets face increased susceptibility to diseases, attributed to factors such as the decline in maternal antibody titers and abrupt alterations in the structure and function of the small intestine (2-4). Suboptimal growth performance in piglets can result in considerable economic setbacks for commercial swine farms (5).

The use of antimicrobial agents stands out as one of the most economically viable approaches for maintaining or improving the health and feed efficiency of animals raised in conventional agricultural methods (6). Among intensive animal production systems, the swine production sector exhibits one of the highest rates of antimicrobial usage, considering both absolute quantity and treatment frequency (7). Antimicrobial agents are frequently added to piglet feed from birth until weaning, with the goal of improving the composition of piglet intestinal microbiota, thereby reducing the potential impact of postweaning diarrhea (4). However, the excessive and improper use of antimicrobial agents in veterinary medicine has resulted in the development of bacteria that are resistant to these antimicrobial agents (7-9). In 2006, the European Union (EU) enforced a prohibition on the utilization of antimicrobial agents as growth promoters (10). This restriction, coupled with the potential for its expansion to other nations, has catalyzed widespread research initiatives dedicated to investigating alternative strategies that can robustly contribute to animal health and performance (4, 11). In Korea, the inclusion of antimicrobial growth promoters in animal feeds was banned starting from July 2011 (5). If proper application proves inadequate, treatment may be extended and involve suboptimal dosing, potentially facilitating the development of bacterial resistance (12).

Several studies have reported an association between the method of antimicrobial administration and the development of resistance (5, 13-16). In pig farms, the oral route of administration is frequently utilized to administer antimicrobial agents to a broad population of animals simultaneously (17). Antimicrobial agents are typically delivered through two primary oral routes: either liquid administering into drinking water or powder administering into feed (5, 17). In pigs, the administration of antimicrobial agents through in-water dosing is applicable in two specific scenarios: metaphylaxis and treatment (5). Metaphylaxis entails the proactive treatment of animal populations experiencing diverse levels of disease before visible manifestations of the disease occur (5).

The objective of a brief dosing regimen, whether administered as a single dose or at regular intervals, is to achieve both a microbiological and clinical cure (5). In instances of disease outbreaks among pigs, the liquid administration of antimicrobial agents via drinking water dosing is employed for a brief duration until clinical signs subside. A successful dosing event must ensure that a substantial proportion of pigs within a group achieve the necessary systemic exposure to the antimicrobial agents, thereby effectively reducing or eliminating the targeted pathogen and achieving a significant level of clinical efficacy (5). Additionally, this approach is crafted to minimize the emergence and dissemination of antimicrobial resistant pathogens. The aim of study was to analyze the effects of liquid administering antimicrobial agents on antimicrobial resistance.

Materials and Methods

Experimental Design

To analyzing effects of liquid administering antimicrobial agents on reducing antimicrobial resistance, we selected 5 pig farms that have both a weaner house with a proportional liquid dispenser (such as Dosatron, Dosatron International, Tresses, France), and a weaner house that dose not have proportional liquid dispenser. We confirmed that weaned piglets in weaner house with proportional liquid dispenser were treated antimicrobial agents via drinking water, and weaned piglets in weaner house without proportional liquid dispenser were treated antimicrobial agents with powder administering into feed.

The fecal and dust samples were collected from weaner house with a proportional liquid dispenser, and a weaner house that does not have one at same time. To collect feces and dust samples, a sterile surgical gauze swab was moistened with 10 mL of sterile phosphate-buffered saline solution. Approximately 10 g of fecal samples were obtained, and two separate areas within pig farms were swabbed to acquire around 10 g of dust samples. All samples were transported

under aseptic conditions to the laboratory at 4°C for the isolation of *Escherichia coli* (*E. coli*) and *Enterococcus* species (spp.).

Isolation of E. coli

Sterilely gathered fecal and dust samples were separately inoculated into 5 mL of mEC (Becton-Dickinson, MD, USA) broth media and incubated at 37°C for a duration of 24 hours. Following the incubation period, mEC medium was streaked to MacConkey (Becton-Dickinson, MD, USA) agar media and subjected to further incubation at 37°C for a duration of 24 hours. Distinctive pink-colored colonies were chosen from each sample, and the confirmation of *E. coli* identification was conducted through polymerase chain reaction, as outlined in a previously described study (18). In this study, a comprehensive examination was conducted on a total of 80 isolates of *E. coli*: 44 from "with proportional liquid dispenser", and 36 from "without proportional liquid dispenser".

Isolation of Enterococcus species

Sterilely gathered fecal and dust samples were separately inoculated into 5 mL of Enterococcossel broth media (Becton-Dickinson, MD, USA) and incubated at 37°C for a duration of 24 hours. Following the incubation period, Enterococcossel medium was streaked to Enterococcossel agar media (Becton-Dickinson, MD, USA) and subjected to further incubation at 37°C for a duration of 24 hours. Distinctive black-colored colonies were chosen from each sample, and the confirmation of *Enterococcus* spp. identification was conducted through polymerase chain reaction, as outlined in a previously described study (18). In this study, a comprehensive examination was conducted on a total of 79 isolates of *Enterococcus* spp.: 39 from "with proportional liquid dispenser", and 40 from "without proportional liquid dispenser".

Antimicrobial Susceptibility Test

Antimicrobial susceptibility of all isolates of *E. coli* and *Enterococcus* spp. was assessed through 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 (19): 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). Each antimicrobial disc used in this study was bought from Becton-Dickinson (MD, USA). Strains exhibiting resistance to three or more CLSI subclasses were categorized as multi-drug resistant isolates (20).

Statistical analysis

Statistical analysis was performed using SPSS version 12.0 software (SPSS, Chicago, Illinois, USA). The evaluation of antimicrobial resistance rates in *E. coli* and *Enterococcus* spp. from pig farms was carried out employing the Chi-square test.

Results

Antimicrobial resistance and Multi-drug resistance of E. coli

Table 1 outlines the antimicrobial resistance profiles of *E. coli* isolates obtained from weaner house with and without proportional liquid dispenser. The resistance rates to ampicillin, streptomycin, oxytetracycline, tetracycline, florfenicol, chloramphenicol, nalidixic acid, ciprofloxacin, sulfisoxazole, and trimethoprim-sulfamethoxazole were more than 50.0%. We found that the resistance rates to chloramphenicol, nalidixic acid, ciprofloxacin, trimethoprim-sulfamethoxazole, and colistin in weaner house with proportional liquid dispenser were lower than that in weaner house without proportional liquid dispenser. Interestingly, the resistance ratio to chloramphenicol was significantly lower in house with liquid dispenser (77.8%) compared to that in house without liquid dispenser (90.9%). There was no difference of multi-drug resistance ratio between weaner house with and without proportional liquid dispenser (Table 2). However, resistant to 9 antimicrobial subclasses, and 10 antimicrobial subclasses were lower in weaner house with proportional liquid dispenser (22.2%, and 2.8%, respectively) compared to without proportional liquid dispenser (43.2%, and 4.5%, respectively).

Table 1. Antimicrobial resistance phenotype of *E. coli* isolated from weaner house with and without proportional liquid dispenser

Antimicrobial subclasses Antimicrobial agents	No. of resistant isolates (Antimicrobial resistance %)*	
	Without proportional liquid dispenser (n=44)	With proportional liquid dispenser (n=36)
Aminopenicillins		
Ampicillin	35 (79.5%)[b]	34 (94.4%)[a]
β-lactam / β-lactamase inhibitor combinations		
Amoxicillin-clavulanic acid	12 (27.3%)	12 (33.3%)
Aminoglycosides		
Gentamicin	15 (34.1%)	15 (41.7%)
Streptomycin	27 (61.4%)[b]	27 (75.0%)[a]
Kanamycin	26 (59.1%)	24 (66.7%)
Tetracyclines		
Oxytetracycline	43 (97.7%)	34 (94.4%)
Tetracycline	43 (97.7%)	34 (94.4%)
Phenicols		
Florfenicol	31 (70.5%)	24 (66.7%)
Chloramphenicol	40 (90.9%)[a]	28 (77.8%)[b]
Quinolones		
Nalidixic acid	35 (79.5%)	27 (75.0%)
Fluoroquinolones		
Ciprofloxacin	28 (63.6%)	20 (55.6%)
Sulfonamides		
Sulfisoxazole	29 (65.9%)	27 (75.0%)
Trimethoprim-sulfamethoxazole	30 (68.2%)	22 (61.1%)
Lipopeptides		
Colistin	1 (2.3%)	0 (0.0%)

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

Table 2. Multi-drug resistance of *E. coli* isolated from weaner house with and without proportional liquid dispenser

No. of resistance	No. of resistant isolates (Antimicrobial resistance %)*	
	Without proportional liquid dispenser (n=44)	With proportional liquid dispenser (n=36)
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 (2.3%)	1 (2.8%)
4 subclasses	2 (4.5%)[a]	0 (0.0%)[b]
5 subclasses	7 (15.9%)[a]	0 (0.0%)[b]
6 subclasses	4 (9.1%)[b]	8 (22.2%)[a]
7 subclasses	2 (4.5%)[b]	7 (19.4%)[a]
8 subclasses	7 (15.9%)[b]	11 (30.6%)[a]
9 subclasses	19 (43.2%)[a]	8 (22.2%)[b]
10 subclasses	2 (4.5%)	1 (2.8%)
Multi-Drug Resistance (≥ 3 subclasses)	44 (100.0%)	36 (100.0%)

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

Antimicrobial resistance and Multi-drug resistance of Enterococcus spp.

Table 3 outlines the antimicrobial resistance profiles of *Enterococcus* spp. isolates obtained from weaner house with and without proportional liquid dispenser. The resistance rates to tylosin, erythromycin, tetracycline, nalidixic acid, florfenicol, chloramphenicol, gentamicin, kanamycin, and streptomycin were more than 50.0%. We found that the resistance rates to penicillin, tylosin, erythromycin, doxycycline, tetracycline, florfenicol, chloramphenicol, and kanamycin in weaner house with proportional liquid dispenser were lower than that in weaner house without proportional liquid dispenser. Among these, isolates from weaned piglets with proportional liquid dispenser showed significantly lower antimicrobial resistance rates to tetracycline (80.0%), florfenicol (77.5%), and kanamycin (87.5%) compared to without proportional liquid dispenser (89.7%, 92.3%, and 97.4%, respectively).

The multi-drug resistance rates are described in Table 4, and isolates from weaner house with proportional liquid dispenser showed significantly lower multi-drug resistance ratio (85.0%) compared to without proportional liquid dispenser (97.4%).

Discussion

In this study, the objective was to analyze the effects of liquid administering antimicrobial agents on antimicrobial resistance. For this, we selected 5 pig farms that have both a weaner house with a proportional liquid dispenser, and a weaner house that dose not have proportional liquid dispenser, and confirmed that weaned piglets in weaner house with proportional liquid dispenser were treated antimicrobial agents via drinking water, and weaned piglets in weaner house without proportional liquid dispenser were treated antimicrobial agents with powder administering into feed. In assessing the antimicrobial resistance of pig farms, *E. coli* and *Enterococcus* spp. were chosen for analysis due to their presence as intestinal commensal bacteria in piglets. Many countries' antimicrobial surveillance systems commonly utilize *E. coli* and *Enterococcus* spp. as reliable indicators for monitoring the overall level of antimicrobial resistance.

Table 3. Antimicrobial resistance phenotype of *Enterococcus* species isolated from weaner house with and without proportional liquid dispenser

Antimicrobial subclasses Antimicrobial agents	No. of resistant isolates (Antimicrobial resistance %)*	
	Without proportional liquid dispenser (n=39)	With proportional liquid dispenser (n=40)
Aminopenicillins		
Ampicillin	1 (2.6%)	1 (2.5%)
Penicillins		
Penicillin	3 (7.7%)	2 (5.0%)
Macrolide		
Tylosin	36 (92.3%)	34 (85.0%)
Erythromycin	36 (92.3%)	34 (85.0%)
Tetracyclines		
Doxycycline	22 (56.4%)	17 (42.5%)
Tetracycline	35 (89.7%)[a]	32 (80.0%)[b]
Tigecycline	0 (0.0%)	0 (0.0%)
Quinolones		
Nalidixic acid	39 (100.0%)	40 (100.0%)
Fluoroquinolones		
Ciprofloxacin	19 (48.7%)[b]	26 (65.0%)[a]
Glycopeptide		
Vancomycin	0 (0.0%)	0 (0.0%)
Phenicols		
Florfenicol	36 (92.3%)[a]	31 (77.5%)[b]
Chloramphenicol	33 (84.6%)	31 (77.5%)
Aminoglycosides		
Gentamicin	20 (51.3%)[b]	26 (65.0%)[a]
Kanamycin	38 (97.4%)[a]	35 (87.5%)[b]
Streptomycin	38 (97.4%)	40 (100.0%)

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

Table 4. Multi-drug resistance of *Enterococcus* species isolated from weaner house with and without proportional liquid dispenser

No. of resistance	No. of resistant isolates (Antimicrobial resistance %)*	
	Without proportional liquid dispenser (n=39)	With proportional liquid dispenser (n=40)
0 subclass	0 (0.0%)	0 (0.0%)
1 subclass	0 (0.0%)	0 (0.0%)
2 subclasses	1 (2.6%)	6 (15.0%)
3 subclasses	1 (2.6%)	4 (10.0%)
4 subclasses	5 (12.8%)	5 (12.5%)
5 subclasses	19 (48.7%)[a]	4 (10.0%)[b]
6 subclasses	12 (30.8%)[b]	20 (50.0%)[a]
7 subclasses	1 (2.6%)	0 (0.0%)
8 subclasses	0 (0.0%)	1 (2.5%)
Multi-Drug Resistance (≥ 3 subclasses)	38 (97.4%)[a]	34 (85.0%)[b]

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

According to Zhang et al., variations in antimicrobial resistance may occur based on the method of administering antimicrobial agents (21). We found that isolates from house with proportional liquid feeding showed a significantly lower antimicrobial resistance rates to chloramphenicol (77.8%) in *E. coli* compared to that from house without proportional liquid dispenser (90.9%), and tetracycline (80.0%), florfenicol (77.5%), and kanamycin (87.5%) in *Enterococcus* spp. compared to those from house without proportional liquid dispenser (89.7%, 92.3%, and 97.4%, respectively). Interestingly, those antimicrobial agents are frequently treated to weaned piglets via drinking water in Korea (7). These findings indicate that administering antimicrobial agents through drinking water might result in a diminished development of resistance to chloramphenicol, tetracycline, florfenicol, and kanamycin within the investigated population. This outcome implies that the method of delivering antimicrobial agents, particularly through drinking water, could contribute to reducing antimicrobial resistance. This underscores the potential advantages of utilizing drinking water as a means of administering antimicrobial agents to mitigate the development of resistance.

We assumed the cause of this phenomena as follows. Diseased piglets may decrease their feed consumption, resulting in suboptimal doses of antimicrobial agents and less effective disease treatment (2, 5). Conversely, by administering antimicrobial agents through liquid, both diseased and healthy weaned piglets can consume sufficient amounts of water for effective disease treatment, eliminating competition for feed intake (2, 5). Administering antimicrobial agents through drinking water promotes efficient disease treatment by ensuring adequate intake for all piglets, irrespective of their health status (2, 5). This stands in contrast to feed administration, where feed intake may be compromised in diseased individuals.

Mitigating the presence of multi-drug resistant bacteria in pigs is essential to prevent the transmission of antimicrobial resistance from livestock to humans (22). Regardless of whether or not a proportional liquid dispenser is installed, there was no difference of multi-drug resistance of *E. coli* however, we found that there was significant decrease in multi-drug resistance rates of *Enterococcus* spp. in this study. *Enterococcus* spp. serve as recognized reservoirs of resistance genes, and their abundance in pig populations raises apprehensions about the possible transmission of resistance to bacteria that impact human health (23). Tackling multi-drug resistance in pigs not only preserves the effectiveness of antimicrobial agents used in veterinary medicine but also crucially minimizes the risk of zoonotic transmission (24, 25). By reducing the prevalence of resistant strains in pig farming, we actively contribute to the overarching goal of maintaining the efficacy of antimicrobial treatments, thereby fostering both animal welfare and public health.

Nevertheless, the degree of this impact differs across various antimicrobial agents, underscoring the intricate nature of antimicrobial resistance development and emphasizing the necessity for thorough investigations into the underlying mechanisms. These results emphasize the critical need for well-informed decisions regarding antimicrobial administration in pig farming to effectively address antimicrobial resistance.

In summary, we posit that the administration of antimicrobial agents through drinking water fosters improved growth performance outcomes, along with efficient and consistent delivery of antimicrobial agents. This method holds promise for mitigating the emergence of antimicrobial resistance. The findings suggest that utilizing drinking water as a means of administering antimicrobial agents is a favorable approach for disease management in pig production.

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