https://doi.org/10.48048/tis.2024.7223

Extended-Spectrum Beta-Lactamase-Producing *Escherichia coli* in Feces and Wastewater Treatment System in Swine Farms, Thailand

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Received: 19 June 2023, Revised: 12 July 2023, Accepted: 19 July 2023, Published: 5 December 2023

Abstract

The antimicrobial resistance (AMR) problem is one that demands urgent interventions to minimize the rapid spread in humans, animals and the environment. Extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-Ec) could spread from livestock farms to environment or circulate in farms via wastewater. This study aimed to quantify and characterize ESBL-Ec in swine feces and wastewater samples collected from 2 conventional swine farms with wastewater treatment systems. Microbiological analysis was performed on fecal and wastewater samples, and the concentration of cefotaxime (CTX)-resistant bacteria, indicative of ESBL-Ec, was determined across different stages of wastewater treatment. Additionally, ESBL-Ec was isolated and identified from fresh feces, swine house wastewater, and treated wastewater from covered lagoons and photosynthetic ponds. Results indicated a higher concentration of CTX-resistant bacteria in the wastewater in the early process of wastewater treatment. However, CTX-resistant bacteria were not detected in the final photosynthetic pond in one of the 2 farms. ESBL-Ec was identified in fresh feces from swine houses and wastewater treatment plants in both farms. Further antimicrobial susceptibility testing revealed diverse resistance patterns among the ESBL-Ec isolates, with tetracycline and gentamicin demonstrated the highest resistance rates. The ESBL genes identified belonged to the CTX-M-14, CTX-M-17 and CTX-M-55. This study demonstrated the presence of ESBL-Ec in swine feces and wastewater, emphasizing the importance of effective wastewater treatment before discharging to limit the spread of AMR bacteria.

Keywords: Antimicrobial resistance, CTX-M genes, Swine farm, Thailand, Wastewater

Introduction

The antimicrobial resistance (AMR) problem is one that demands urgent interventions to minimize the rapid spread in humans, animals, and the environment. The interconnectedness of the humans, food-producing animals, and environment has played a role in driving AMR spread from 1 sector to another [1]. As a result, WHO suggested the One Health approach in tackling the AMR debacle. Besides the directive that member states should develop a national action plan, WHO in 2021 has implemented the global surveillance including standard methodology in human, food chain and environmental which seek to quantify extended-spectrum beta-lactamase-producing *Escherichia coli* (ESBL-Ec) to provide information for making the most contributions that warrant urgent preventive controls [2]. The detection and quantification of ESBL-Ec in hotspot sources, such as rivers receiving wastewater from human sewage, and wastewater influenced by animal waste from food-producing animal farms may provide in-depth understanding of AMR path of dissemination [2].

Pigs are produced in large quantities to meet the high demand of pork in some Asian countries [3]. As in the case of other food-producing animals, antimicrobial agents are routinely used in swine production for controlling the spread of infections, and prevention of infection [4]. In Thailand, the use of antimicrobials to control infectious diseases in food-producing animals is often conducted without appropriate supervision by veterinarians [5]. Undoubtedly, this practice has led to the emergence of AMR

in most bacteria isolated from food-producing animals including pigs [6]. The unfortunate incident is that AMR bacteria from food-producing animal farms are contaminate the environment through wastewater flowing from the farms [7]. Although wastewater treatment plants are available on some swine farms in Thailand, there is no regulation that limits the discharge of AMR bacteria or antimicrobial residues released into the environment. As reported previously, several bacteria resistant to powerful antimicrobial agents such as third generation cephalosporin are isolated from swine feces as well as wastewater from those farms [8-10]. The presence of clinically relevant resistant bacteria in food-producing animals threatens the sustainability of animal production and human health and warrants further investigation.

ESBLs are widely spreading enzymes that pose a significant challenge in the treatment of infections. They are capable of deactivating ESBLs such as cephalosporins, monobactam but are inhibited by clavulanic acid, sulbactam, and tazobactam [11]. Additionally, the genes responsible for ESBL are mostly found on plasmids that are horizontally transferred among bacterial species. Members of the *Enterobacteriaceae* family, especially *Escherichia coli,* frequently bear diverse ESBLs [11]. In Thailand, several studies have shown the presence of ESBL producer isolated from food-producing animals [8, 10]. Although these studies have indicated the occurrence of ESBL producer circulating in food-producing animal farms, the actual quantity of ESBL producer in wastewater treatment plant in food-producing animal farm is rarely investigated. Therefore, the purpose of this study was to quantify and determine the characteristics of ESBL-Ec in swine feces and wastewater from swine farms.

Material and methods

Study site

In this study, samples were taken from 2 conventional swine farms identified as Farm A and Farm B having a capacity of 150 fattening pigs/house with an area of 6 m^2/pig. The settings on these farms are similar with each farm consisting of swine house(s), cover lagoon (for anaerobic condition of treating wastewater) and photosynthetic pond(s) (aerobic condition of treating wastewater). Wastewater generated as a result of cleaning the swine house(s) are released to a covered lagoon for treatment after which it is released to the first and second photosynthetic ponds for further treatment (**Figure 1**).

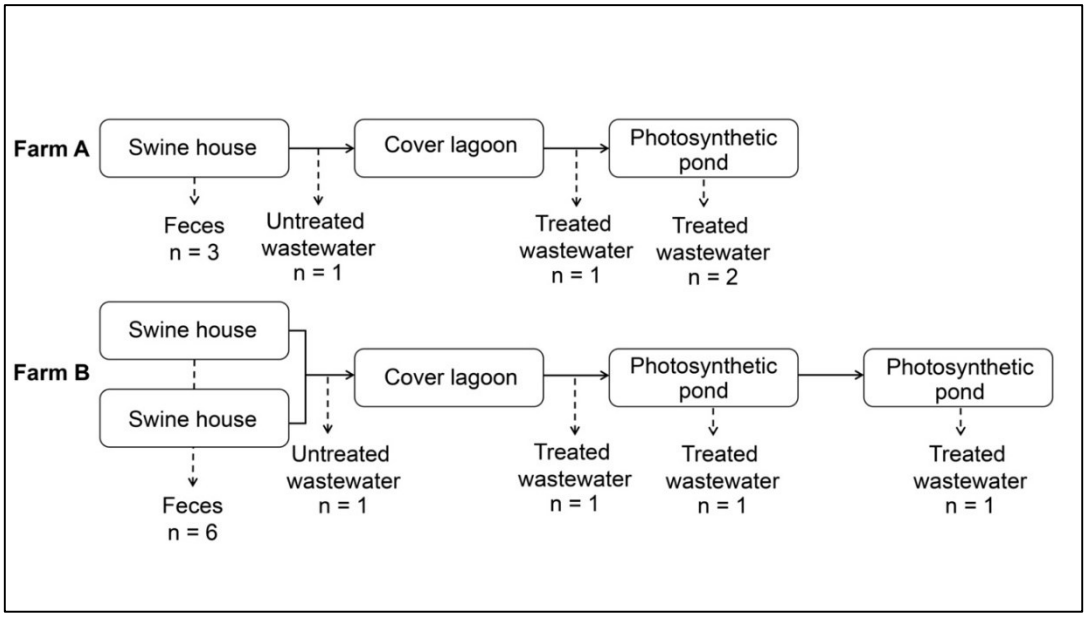

Figure 1 Type and number of samples in each sampling location and the process of wastewater treatment in swine farms. n, number of samples.

Sample collection and preparation

Two conventional swine farms with wastewater treatment system were purposely selected for the detection of ESBL-Ec. Nine fecal samples and 8 wastewater samples (farm A, 3 fecal and 4 wastewater samples; farm B, 6 fecal and 4 wastewater samples) were collected in October 2021 as shown in **Figure 1**. A pooled fecal sample was designed to collect from head, middle, and end zone of swine house. Fecal samples aseptically collected from apparently healthy swine were pooled into sterile tubes for

microbiological examination. For wastewater, sterile bottles were used to collect wastewater (volume of 500 mL) from 3 point (at least one sample/point/farm) namely: Untreated wastewater (influent) from an open distribution line of swine houses (n = 2), treated wastewater (effluent) from covered lagoons (n = 2), treated wastewater (effluent) in photosynthetic ponds (n = 4). The samples were subjected to microbiological procedure for detection of ESBL-Ec. All samples were immediately transported on ice to the laboratory for further analysis.

Bacterial isolation from fecal samples

Pooled fecal samples were streaked using a sterile cotton swab onto MacConkey agar (MAC) (Oxoid, Ltd, Hamshire, UK) plates and then on MAC supplemented with 1 µg/mL cefotaxime (Sigma-Aldrich, USA) (MAC-CTX) Agar. Plates were then aerobically incubated at 37 °C for 16 - 18 h. After incubation, a maximum of 5 pink colonies suspected to be *E. coli* were randomly picked from each plate. A bacterial stock culture with 10 % glycerol was made and stored at −80 °C for further analysis. This study was approved by the Ethics Committee for Animal Research and Welfare of Masarakham University, Thailand (license number: IACUC-MSU-18/2020).

Bacterial isolation from wastewater samples

Firstly, each wastewater samples collected (volume of 500 mL) were subjected to filtration using sterile filter paper to remove large particles. Then after 100 mL of the filtrate was further filtered using 0.2 µm pore size, 25 mm diameter, cellulose nitrate membrane filter (Whatman™, Cytiva, Germany). After the water drained, the filter was then taken using sterile forceps and placed upside down on MAC and then on MAC-CTX. Finally, the filter was placed in tryptic soy broth (TSB) (Becton Dickinson and company, Sparks, MD, USA) enrichment media supplemented with 1 µg/mL of CTX (TSB-CTX). All the culture plates and tubes were aerobically incubated at 37 °C for 16 - 18 h. For samples inoculated directly on MAC-CTX, a maximum of 5 isolates with pink coloration suspected to be *E. coli* were picked. However, for samples in TSB-CTX, a loop of bacterial suspension taken from the enrichment media was streaked on MAC-CTX Agar plates and incubated as described above in order to isolate distinctive colonies. After incubation, a maximum of 5 pink colonies suspected to be *E. coli* were randomly picked from each plate and stored as described above for further analysis.

Bacterial identification

Identification of isolates was performed by morphological characteristics, and biochemical tests (indole, catalase, oxidase, urease, Triple Sugar Iron, Methyl-Red-Voges-Proskauer and citrate). Further identification to the species level was performed by polymerase chain reaction (PCR) using primers previously reported [12].

Enumeration of bacteria from wastewater samples

To enumerate the CTX-resistant bacteria present in wastewater, wastewater samples were subjected to a 2-fold serial dilution using 0.85 % NaCl (2^{-1}, 2^{-2}, 2^{-3}, 2^{-4}). Thereafter, 100 µl aliquot was spread on MAC-CTX and incubated at 37 °C for 16 - 18 h. The pink colonies formed that are presumable *E. coli* were counted.

Antimicrobial susceptibility testing

Minimum inhibitory zone of antimicrobials for CTX-resistant *E. coli* isolates were determined by the disc diffusion method following the Clinical and Laboratory Standard Institute guidelines (CLSI) [13], using 8 antimicrobial discs (Oxoid, Ltd, Hamshire, UK). The following 8 antimicrobial agents were tested: CTX (30 µg/disc), chloramphenicol (CHL, 30 µg/disc), ciprofloxacin (CIP, 5 µg/disc), gentamicin (GEN, 10 µg/disc), meropenem (MER, 10 µg/disc), nalidixic acid (NAL, 30 µg/disc), tetracycline (TET, 30 µg/disc), and sulfamethoxazole/trimethoprim (SXT, 23.75/1.25 µg/disc). Resistance breakpoints, for the 8 antimicrobial agents tested were interpreted following the CLSI guidelines [14]. Additionally, to investigate ESBL production among the CTX-resistant isolates, a double-disc synergy test using clavulanate/amoxicillin (10/20 µg/disc), CTX (30 µg/disc), ceftazidime (30 µg/disc), and cefpodoxime sodium (10 µg/disc) was conducted as previously described [15].

ESBL genes identification

ESBL genes were identified by performing multiplex PCR using primers reported previously [16]. The subtypes of the CTX-M-1-group and CTX-M-9-group beta-lactamases were determined by DNA sequencing based on AMR patterns and sampling locations using previously reported primer pairs [17-18].

Both strands of the amplified DNA fragments were sequenced at the Solgent Anaysis Services, Korea. The encoded amino acid sequences were analyzed using the BLAST program (National Center for Biotechnology Information, Bethesda, MD, USA).

Results and discussion

Quantification of CTX-resistant bacteria

This study indicated that the concentration of CTX-resistant bacteria in the swine feces and wastewater varied among farms and stages of the wastewater treatment process (**Table 1**). Concentration of CTX-resistant bacteria in wastewater from swine house was approximately 10 folds high in Farm A than in Farm B. Additionally, concentration in treated wastewater outlet from covered lagoon was 7,480 CFU/mL (3.87 $\log_{10}$ CFU/mL) and 2,980 CFU/mL (3.47 $\log_{10}$ CFU/mL) in Farm A and Farm B respectively. This wide variation in concentration of CTX-resistant bacteria between the 2 farms may indicate a potential difference in antimicrobial exposure between the 2 farms. Also, this depicts the transmission of AMR bacteria from swine feces to the wastewater generated in the swine houses. Evidently, the use of antimicrobials in animal production contributed to AMR bacteria emergence [5]. Unexpectedly, the treated wastewater from covered lagoon has higher concentration of CTX-resistant bacteria than untreated wastewater from swine houses in both farms. This finding raises concerns about the effectiveness of the treatment process, suggesting the possibility of an external source of CTX-resistant bacteria such as soil slurry storage surrounding cover lagoons contaminating the treated wastewater [19]. Further investigation and improvement of the treatment processes are necessary to mitigate the spread of CTX-resistant bacteria. In contrast, CTX-resistant bacteria concentration in wastewater in last photosynthetic pond was low on farm A and not detected in treated wastewater in the last photosynthetic pond of farm B (**Table 1**). The efficiency of removal by log scale ranges from 3.43 (wastewater outlet from swine houses) to 1.06 (treated wastewater in the last photosynthetic pond). This suggests that the treatment process in the photosynthetic ponds was effective in reducing the concentration of CTX-resistant bacteria to minimal levels. In accordance with a prior study, bacterial load in wastewater decreases as it moves through the various phases of sewage treatment [20]. Thus, effective treatment of wastewater from food-producing animal farms is an essential intervention that may reduce the AMR bacteria spread.

Table 1 Enumeration of CTX-resistant bacteria of raw wastewater from pig houses and effluent wastewater from pounds and removal capacity of wastewater treatment pond.

Collecting sites	Concentration of CTX-resistant bacteria ($\log_{10}$ CFU/mL)		
	Farm A	Farm B	Average
Wastewater outlet from pig house	3.69	2.72	3.43
Treated wastewater outlet from cover lagoon	3.87	3.47	3.72
Treated wastewater in photosynthetic pond 1	2.67	0	2.37
Treated wastewater in photosynthetic pond 2	None	0	0
Log_{10} reduction[1*]	1.20 (3.87 - 2.67)	3.47 (3.47 - 0)	1.35 (3.72 - 2.37)
Log_{10} reduction[2*]	1.02 (3.69 - 2.67)	2.72 (2.72 - 0)	1.06 (3.43 - 2.37)

Note: [*]Log_{10} reduction[1], Log_{10} (concentration of CTX-resistant bacteria at treated wastewater outlet from cover lagoon) –Log_{10} (concentration of CTX-resistant bacteria in wastewater of the last photosynthetic pound); Log_{10} reduction[2], Log_{10} (concentration of CTX-resistant bacteria in wastewater from pig house) −Log_{10} (concentration of CTX-resistant bacteria in wastewater of the last photosynthetic pound)

Enumeration and antibiogram of ESBL-Ec isolates

Presence of 43 ESBL-Ec isolates was investigated in fresh feces and wastewater collected from swine house. Additionally, treated wastewater collected from covered lagoon and photosynthetic ponds were also examined for ESBL-Ec. Specifically, of the 21 ESBL-Ec isolated from Farm A, 23.8 % (5/21 isolates) were from fresh feces, 28.6 % (6/21 isolates) from wastewater from a swine house, 23.8 % (5/21 isolates) from treated wastewater in covered lagoon, 23.8 % (5/21 isolates) from treated wastewater in a photosynthetic

pond. On Farm B, of the 22 ESBL-Ec, 22.7 % (5/22 isolates) were isolated from swine feces, 18.2 % (4/22 isolates) from wastewater from swine house, 45.5 % (10/22 isolates) from treated wastewater from covered lagoon, and 13.6 % (6/22 isolates) from treated wastewater in the first photosynthetic pond. The presence of ESBL-Ec is not limited to a particular stage of the wastewater treatment process but ESBL-Ec was detected in the initial fresh feces as well as in the wastewater from swine houses and the treated wastewater in both covered lagoon and photosynthetic pond. Thus, the presence of ESBL-Ec in fresh feces and throughout the treatment process raises concerns about the transmission of AMR genes from livestock to the environment.

Table 2 presents the number of isolates in interpretive categories of susceptible, intermediate, and resistance from the zone diameter breakpoints (mm) of each antimicrobial agent. ESBL-Ec showed the highest rate susceptible to MEM (100.0 %), followed by CIP (60.6 %), NAL (54.6 %), SXT (36.4 %), GEN (30.3 %), CHL (18.2 %), and TET (12.1 %). However, the ESBL-Ec identified also showed the highest resistance to TET (87.9 %), followed by GEN (69.7 %), SXT (60.6 %), NAL (36.4 %), CHL (36.4 %), and CIP (24.2 %). Furthermore, of the 43 ESBL-Ec isolates, 38 isolates (88.7 %) were multidrug resistant (7, 4.7 % (2/43); 6, 16.3 % (7/43); 5, 11.6 % (5/43), 4, 34.9 % (15/43); and 3 drug groups, 20.9 % (9/43)) and the remaining 5 isolates (11.6 %) were resistant to 2 classes of antimicrobial agents (**Table 3**). The ESBL-Ec identified showed diverse resistance to more than 3 classes of antimicrobial agents making them multidrug resistant (MDR). Although the veterinarians of both swine farms in this study used amoxicillin for treating unhealthy pigs, the ESBL-Ec isolates were found in healthy pigs with high percentage of resistance to TET, GEN, and SXT, and followed by NAL, CHL, and CIP, indicating the spread of AMR commensal bacteria from recovered animals to other animals in the same house. A previous study in Thailand also reported a high percentage of ESBL-Ec from swine feces were MDR [21]. Moreover, widespread use of antimicrobials such as TET, and GEN in animal production in Thailand may result in the high AMR rate [22]. Additionally, ESBL-Ec are mostly reported to harbor plasmids that carry genes that confer resistance to other antimicrobial classes such as aminoglycosides, and fluoroquinolone [23], and this phenomenon may explain the characteristic MDR in the ESBL-Ec. Conversely, all the ESBL-Ec were susceptible to MEM which is a useful finding from a public health perspective because carbapenem (e.g. MEM) is a drug of choice for treating serious ESBL bacterial infections [24]. It is therefore important to strictly regulate carbapenem usage in food animal production in Thailand in order to prevent emergence of carbapenem-resistant bacteria. Hence, the presence of ESBL-Ec resistant to third generation cephalosporins with several MDR in swine farms may further serve the occurrence and circulation of diverse resistance of more antimicrobial classes in farms.

Extended-spectrum beta-lactamase genes associated with ESBL-Ec

In order to fully acknowledge the phenotypic resistance observed in the ESBL-Ec, the associated resistance genes were investigated (**Table 3**). All 5 isolates of ESBL-Ec isolated from feces collected from swine Farm A bears CTX-M-9 family of beta-lactamase genes ($bla_{\text{CTX-M-9 gr.}}$) However, ESBL-Ec bearing CTX-M-1 family of beta-lactamase genes ($bla_{\text{CTX-M-1 gr.}}$) (3 out of 5 isolates) and $bla_{\text{CTX-M-9 gr.}}$ (2 out of 5 isolates) were isolated from feces collected from Farm B. Additionally, as shown in **Table 3**, all 33 ESBL-Ec isolates bearing $bla_{\text{CTX-M-1 gr.}}$ (17 isolates), and $bla_{\text{CTX-M-9 gr.}}$ (16 isolates) originated from wastewater from both farms. ESBL-Ec isolates (53.49 %, 23/43 isolates) harboring CTX-Ms and TEMs genes were also identified. In the present study, ESBL genes identified belong to CTX-M-1 group, and CTX-M-9 group as observed in a previous study conducted in swine in Thailand [25]. The presence of resistance genes on the chromosome or on plasmids of resistant bacteria is a major contributor to antimicrobial resistance in bacteria [26]. Furthermore, 16 isolates of the 43 ESBL-Ec selected for subtyping reveals that, 7 were $bla_{\text{CTX-M-55}}$, 7 were $bla_{\text{CTX-M-14}}$ and 2 were $bla_{\text{CTX-M-17}}$. The CTX-M-55-producing *E. coli* and CTX-M-14-producing *E. coli* were detected at all sampling locations on the swine farms. Result corresponds favorably with several studies conducted in swine in Thailand where $bla_{\text{CTX-M-55,}}$ and $bla_{\text{CTX-M-14,}}$ were the predominant ESBL gene identified [25,27,28]. Enzymes in these sub-families are widely disseminated and frequently reported [29].

Table 2 Antibiogram of ESBL-Ec isolates from wastewater samples collected from wastewater system in swine farm A and B.

ATM	Number of isolates (%)			Number of ESBL-producing bacterial isolates in each inhibition zones: diameters (mm)																					
	R	I	S	0	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	> 25	Total
SXT	20 (60.6)	1 (3.0)	12 (36.4)	20									1					1	1		1		1	8	33
TET	29 (87.9)	0	4 (12.1)	7		5	5	10	2												1		1	2	33
MER	0	0	33 (100)																					33	33
GEN	23 (69.7)	0	10 (30.3)	16	4	1		1		1			2	1	1	6									33
NAL	12 (36.4)	3 (9.1)	18 (54.6)	10						2				1	1	1	1	1	1	1	2	4	7	1	33
CHL	12 (36.4)	15 (45.5)	6 (18.2)	1		4		3	1	3	7	3	3	2							1		1	4	33
CIP	8 (24.2)	5 (15.2)	20 (60.6)	3					2	2	1			1		2	1	1		1	1	1		17	33

Note: ATM, Antimicrobials; R, Resistance; I, Intermediate; S, Susceptible; SXT, Sulfamethoxazole/trimethoprim; TET, Tetracycline; MER, Meropenem; GEN, Gentamycin; NAL, Nalidixic acid; CHL, Chloramphenicol; CIP, Ciprofloxacin; gray box, resistance; slight gray box, intermediate; and white box, susceptible.

Table 3 Antimicrobial resistance patterns of ESBL-Ec and their ESBL genes isolated from fresh feces and wastewater collecting point from each location of swine farm A and B.

Collecting location	Antimicrobial resistance patterns	Subfamilies of CTX-β-lactamase genes (Farm)	No. of isolates	Subtyping of *bla*$_{\text{CTX-Ms}}$*
Fresh feces from pig house	CTX-GEN-TET-NAL-CIP-SXT-CHL	*bla*$_{\text{CTX-M-1gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	*bla*$_{\text{CTX-M-55}}$
	CTX-GEN-TET-NAL-SXT-CHL	*bla*$_{\text{CTX-M-1gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	nd
	CTX-GEN-TET-SXT	*bla*$_{\text{CTX-M-9 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	1	nd
		bla$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	nd
	CTX-GEN-NAL-CIP	*bla*$_{\text{CTX-M-9 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	4	*bla*$_{\text{CTX-M-17}}$
	CTX-GEN-NAL	*bla*$_{\text{CTX-M-9 gr.}}$ (B)	1	*bla*$_{\text{CTX-M-14}}$
	CTX-GEN	*bla*$_{\text{CTX-M-9 gr.}}$ (B)	1	nd
	Subtotal		10	3
Wastewater outlet from pig house	CTX-GEN-TET-NAL-CHL	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	1	nd
	CTX-GEN-TET-NAL-CIP	*bla*$_{\text{CTX-M-9 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	1	*bla*$_{\text{CTX-M-17}}$
	CTX-GEN-TET-CHL	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	2	*bla*$_{\text{CTX-M-14}}$
	CTX-GEN-TET-SXT	*bla*$_{\text{CTX-M-9 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	*bla*$_{\text{CTX-M-14}}$
	CTX-GEN-TET	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	1	nd
	CTX-TET-SXT	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	1	*bla*$_{\text{CTX-M-55}}$
		bla$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	*bla*$_{\text{CTX-M-55}}$
	CTX-TET-NAL	*bla*$_{\text{CTX-M-9 gr.}}$ (B)	1	nd
	CTX-GEN	*bla*$_{\text{CTX-M-1 gr.}}$ (B)	1	nd
	Subtotal		10	5
Treated wastewater outlet from cover lagoon	CTX-GEN-TET-NAL-CIP-SXT	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	4	*bla*$_{\text{CTX-M-55}}$
	CTX-GEN-TET-SXT-CHL	*bla*$_{\text{CTX-M-1 gr.}}$ (B)	1	nd

Collecting location	Antimicrobial resistance patterns	Subfamilies of CTX-β-lactamase genes (Farm)	No. of isolates	Subtyping of *bla*$_{\text{CTX-Ms}}$*
	CTX-TET-NAL-SXT-CHL	*bla*$_{\text{CTX-M-1 gr.}}$ (A)	1	*bla*$_{\text{CTX-M-55}}$
	CTX-GEN-TET-CHL	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	2	*bla*$_{\text{CTX-M-14}}$
	CTX-GEN-TET-SXT	*bla*$_{\text{CTX-M-9 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	3	*bla*$_{\text{CTX-M-14}}$
	CTX-GEN-CHL	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	1	nd
	CTX-GEN-TET	*bla*$_{\text{CTX-M-9 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	1	nd
	CTX-TET	*bla*$_{\text{CTX-M-1 gr.}}$ (B)	2	nd
	Subtotal		15	4
Treated wastewater in photosynthetic pond	CTX-GEN-TET-NAL-CIP-SXT-CHL	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	*bla*$_{\text{CTX-M-55}}$
	CTX-GEN-TET-NAL-CIP-SXT	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	nd
	CTX-TET-NAL-CIP-SXT-CHL	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (B)	1	nd
	CTX-GEN-TET-NAL-SXT	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	1	nd
	CTX-GEN-SXT-CHL	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	1	*bla*$_{\text{CTX-M-14}}$
	CTX-TET-SXT	*bla*$_{\text{CTX-M-1 gr.}}$, *bla*$_{\text{TEMs}}$ (A)	2	*bla*$_{\text{CTX-M-55}}$
	CTX-SXT	*bla*$_{\text{CTX-M-9 gr.}}$ (A)	1	*bla*$_{\text{CTX-M-14}}$
	Subtotal		8	4
Total isolates			43	16

Note: *Some isolates of each subfamily of CTX-β-lactamase genes from both farms were randomly selected to subtype; nd, not determined; CTX, Cefotaxime; GEN, Gentamycin; TET, Tetracycline; NAL, Nalidixic acid; CIP, Ciprofloxacin; SXT, Sulfamethoxazole/trimethoprim; CHL, Chloramphenicol

In previous study, it showed the persistence of one of 3 AMR plasmids in bacteria isolated from broiler farm after the introduction of AMR bacteria to farms via new day-old chicks [30]. Similarly, present results revealed that ESBL-Ec isolates bearing the same ESBL gene (*bla*$_{\text{CTX-M-14}}$ or *bla*$_{\text{CTX-M-55}}$) showing same AMR pattern was found in more than 1 process of wastewater treatment and/or animal feces (**Table 3**) indicating that AMR bacteria from wastewater treatment system may circulate in farm environment and infect new swine. Despite ESBL-Ec bearing *bla*$_{\text{CTX-M-17}}$ was found in swine feces and raw wastewater but undetected in wastewater treatment process, it is important to note that these ESBL genes are located on mobile genetic elements, such as plasmids, facilitating their transfer to other bacteria and contributing to the persistence of AMR bacteria in the wastewater treatment system. The aforementioned ESBL genes are identified on mobile genetic elements such as plasmids [25,31]. Thus, current results indicated that not only farm animals are reservoir of AMR bacteria, but also the wastewater treatment system as the report of high prevalence (88.7 %, 133/150 dairy farms) of ESBL-Ec isolated from wastewater collecting from manhole in dairy farms, Thailand [32]. However, this present study reveals that the process of wastewater treatment in Thailand swine farm may reduce AMR bacterial load as same as the previous study that showed the high treatment efficiency of *E. coli* reduction due to wastewater treatment plant of industies and hospitals, South Africa [33]. Therefore, this study indicated that livestock farms must set a potential wastewater treatment plant to ensure the efficient reduction of bacterial load as well as AMR bacteria before released to environment.

Conclusions

Present study showed the occurrence of ESBL-Ec in feces and wastewater in swine farms. Although concentration of CTX-resistant bacteria was low in the last steps of wastewater treatment system, wastewater can serve as a medium for transmitting resistant bacteria from swine farms to environments. As the bacteria with the same AMR pattern and ESBL genes was found in many steps of wastewater treatment process, wastewater could play an important role of the circulating of AMR bacteria in animal farms.

Further studies should assess that ESBL plasmid carried bacteria isolated from wastewater treatment system could transfer to new swine and persist in the farms by plasmid analysis.

Acknowledgements

This research project was financially supported by Mahasarakham University, Thailand. We would like to thank Dr. Vajara Wipassa for his support in sample collection and molecular identification technique. We are grateful to Veterinary Infectious Disease Research Unit (VIDRU), Thailand.

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