Antimicrobial Resistance Profiles of *Escherichia coli* Isolated from Multiple Organs of Diseased Swine in Nakhon Pathom Province, Thailand

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Abstract

This study aimed to determine antimicrobial resistance profiles of *Escherichia coli* isolated from multiple organ samples of diseased swine in Nakhon Pathom province, Thailand. Antimicrobial susceptibility of *E. coli* against 13 antimicrobial agents was tested by a disk diffusion method. A total of 119 isolates from multiple organs, including intestines (n=45), tonsils (n=32), lungs (n=14), colon swabs (n=10), mesenteric lymph nodes (MSLN) (n=10), nasal swabs (n=4), oral swabs (n=3), and tracheobronchial lymph nodes (TBLN) (n=1) of diseased swine were examined. As a result, antimicrobial resistant *E. coli* isolates were detected from all organ samples. The majority of *E. coli* isolates resisted to AMP 96.64% (115/119), TE 92.44% (110/119), SXT 81.51% (97/119), C 79.83% (95/119), CN 64.71% (77/119) and CIP 62.18% (74/119), respectively. Almost 50% of studied *E. coli* isolates resisted to CTX 47.90% (57/119) and CRO 42.86% (51/119) and only one-third of studied *E. coli* isolates resisted to AMC 11.76% (14/119), CT 11.76% (14/119) and CAZ 10.08% (12/119). Distribution of detected antimicrobial resistant *E. coli* in each organ was not obviously different. However, samples from intestines, tonsils, lungs and MSLN possessed isolates resisting to at least one antimicrobial agent. In addition, the most frequent patterns of multidrug resistance (MDR) were AMP-CRO-CTX-CIP-NA-C-CN-TE-SXT 12.61% (15/119) and AMP-CIP-NA-C-CN-TE-SXT 12.61% (15/119). In summary, a large number of antimicrobial resistant *E. coli* isolated from diseased swine in Nakhon Pathom province was observed. This study highlighted that antimicrobial resistant *E. coli* were not only present in the intestinal tract, but also in systemic organs of swine.

Keywords: *Escherichia coli, Antimicrobial resistance, Swine*

1. Introduction

Pathogenic *Escherichia coli* has been the leading cause of diarrhea in swine causing economic loss in worldwide swine farming (Urairong, 1992). In Thailand, a previous report showed that 48% piglets were sick from diarrhea with 50 – 90% mortality rate. In general, farmers use antimicrobial agents for therapeutics and producing growth promoter for swine (Sooksai et al., 2016). In Thailand, common antimicrobial agents that were used in swine farms include amoxicillin (AML), ampicillin (AMP), enrofloxacin (ENR), tetracycline (TE), oxytetracycline (OT), gentamicin (CN), neomycin (N), ceftriaxone (CRO) and colistin (CT). These antimicrobial agents were mixed in animals feed to prevent bacterial infection as well as to help animals digest food by using sub-therapeutic dose. These procedures can induce emergence of antimicrobial resistant *E. coli* in swine and farm environment (Gibbons et al., 2016).

In addition, swine can serve as a reservoir of multiple drug resistant (MDR) *E. coli* and is a potential route of transmission to humans (Reid et al., 2017). A previous study showed that MDR *E. coli* isolates were detected in fecal samples (78%) of swine in the farms located in Northern Thailand (Love et al., 2015). *E. coli* isolates often resisted to 4 classes of antimicrobial agents including penicillins, phenicols, tetracyclines and sulfonamides and the most common reported MDR pattern in *E. coli* was AMP-C-S-SXT-TET. Furthermore, previous research demonstrated that isolated *E. coli* from different organs of swine in Northern Thailand were TE resistant (93.8%), AML resistant (89.5%) and AMP resistant (81.8%) (Wongchanthong & Onwan, 2012). However, most reports investigated in intestinal *E. coli*. To date, little is known about antimicrobial susceptibility of *E. coli* isolated from other systemic organs i.e. tonsils, lungs, lymph nodes, etc. This study aimed to determine antimicrobial resistance profiles of *E. coli* isolated from multiple organ samples of diseased swine in Nakhon Pathom province, Thailand. Outcome of the present

study revealed prevalence of antimicrobial resistant *E. coli* from intestinal and extra-intestinal organs of diseased swine.

2. Objectives

1. To determine antimicrobial susceptibility of *E. coli* isolated from multiple organs of diseased swine in Nakhon Pathom province, Thailand by a disk diffusion method
2. To study distribution of antimicrobial resistant *E. coli* isolates from individual organs of diseased swine in Nakhon Pathom province, Thailand
3. To determine MDR pattern of *E. coli* isolated from diseased swine in Nakhon Pathom province, Thailand

3. Materials and Methods

3.1 Sample collection

A total of 149 samples from organ tissue of diseased swine were obtained from 16 farms in Nakhon Pathom province, Thailand. Obtained samples composed of 3 organ systems: Respiratory system i.e. lungs (n=49) and nasal swabs (n=12); Immune system i.e. tonsils (n=22), mesenteric lymph nodes (MSLN) (n=12) and tracheobronchial lymph nodes (TBLN) (n=11); and Digestive system i.e. intestines (n=23), oral swabs (n=12), colon swabs (n=7) and liver (n=1). All samples were kindly provided by Veterinary practitioners and the isolation of *E. coli* from each sample were previously performed by a laboratory technician at Kamphaengsaean Veterinary Diagnostic Center, Faculty of Veterinary Medicine, Kasetsart University, Kamphaengsaean, Thailand. *E. coli* isolates were identified by selective media MacConkey agar (Oxoid, UK) and biochemical tests including Triple sugar iron and IMViC (Indole, Methyl Red, Voges Proskauer and Simmon's citrate test). Isolated *E. coli* were stocked in 20% glycerol with brain heart infusion (BHI) and kept in -80°C until use.

3.2 Antimicrobial susceptibility test

E. coli isolates from 20% glycerol with BHI stock in -80°C were restreaked onto MacConkey agar (Oxoid, UK) and incubated at 37 °C for 24 hours (hrs). After incubation, a single *E. coli* colony was transferred into 3 mL Mueller Hinton broth (MHB) and further incubated at 37 °C for 3 - 5 hrs. The second 3 mL MHB tube was used to adjust turbidity to 0.5 McFarland by the Grant bio DEN-1 densitometer. Next, a sterile cotton swab was dipped into bacterial suspension in the second MHB tube and spread over the entire surface of the Mueller Hinton agar (MHA) plate. Total 2 inoculated MHA plates were prepared for each isolate.

A total of 13 antimicrobial disks (Oxoid, UK) from 9 antimicrobial classes which were described by Clinical and Laboratory Standards Institute guidelines (CLSI, 2016) were used in the present study. They used antimicrobial agents 9 classes namely: 1. Penicillins including AMP (10 µg) and Amoxicillin/clavulanic acid (AMC, 20/10 µg); 2. Cephalosporins including Ceftazidime (CAZ, 30 µg), CRO (30 µg) and Cefotaxime (CTX, 30 µg); 3. Quinolones and fluorquinolones including Ciprofloxacin (CIP, 5 µg) and Nalidixic acid (NA, 30 µg); 4. Polymyxins including CT (10 µg); 5. Phenicols including Choramphenicol (C, 30 µg); 6. Fosfomycins including Fosfomycin (FOT, 200 µg); 7. Aminoglycosides including CN (10 µg), 8.) Tetracyclines including TE (30 µg); and 9. Sulfonamides including Trimthoprim/Sulfamethoxazole (SXT, 1.25/23.75 µg). *E. coli* ATCC® 25922 was used as a quality control strain for antimicrobial susceptibility testing.

Antimicrobial disk (Oxoid, UK) was individually dispensed on 2 inoculated MHA plates by Disk Dispenser (Oxoid, UK). The 6 different antimicrobial disks (AMP, AMC, CAZ, CRO, CTX and CIP) were dispensed on the surface of the first inoculated MHA plate, and other 6 different antimicrobial disks (NA, CT, C, FOT, CN and TE) were applied on the second inoculated MHA plate. After that, sterile forceps were used to transfer the last of antimicrobial disk (SXT) to the center of the second MHA plate. All inoculated MHA plates with an antimicrobial disk on the surface were incubated at 37 °C for 16 - 18 hrs.

Assessment of antimicrobial susceptibility was performed according to the standard guidelines (CLSI, 2016). Antimicrobial resistance (R) was measured by distance at the edge of the zone of inhibited

growth. Each tested antimicrobial agent has different criteria to measure the clear zone distance as the following; AMP (≤ 13), AMC (≤ 13), CAZ (≤ 17), CRO (≤ 19), CTX (≤ 22), CIP (≤ 15), NA (≤ 13), CT (≤ 11), C ≤ 12), FOT (≤ 12), CN (≤ 12), TE (≤ 11) and SXT (≤ 10). The assessment of MDR isolate was justified by resistance to at least 3 different classes of tested antimicrobial agents (Falagas & Karageorgopoulos, 2008).

3.3 Data analysis

Descriptive statistics was used to describe antimicrobial resistance of *E. coli* isolates by percentages. The results included isolation of *E. coli* from multiple organs of swine and antimicrobial resistant profiles of isolated *E. coli* against 13 antimicrobial agents.

4. Results

4.1 Isolation of *Escherichia coli*

Of 149 obtained samples, 51 samples (34.23%; 51/149) were positive for *E. coli* (Table 1). Total 119 *E. coli* isolates were recovered from intestines (37.82%; 45/119), tonsils (26.89%; 32/119), lungs (11.76%; 14/119), MSLN (8.40%; 10/119), nasal swabs (3.36%; 4/119), oral swabs (2.52%; 3/119) and TBLN (0.84%; 1/119). The liver was the only one organ sample that *E. coli* was not detected.

Table 1 The number and percentage of samples and *Escherichia coli* isolates from diseased swine.

Samples	Number of samples	Number of *E. coli* positive samples (%)	Number of *E. coli* isolates (%)
Digestive system			
Intestine	23	16 (69.57)	45 (37.82)
Oral swab	12	2 (16.67)	3 (2.52)
Colon swab	7	5 (71.43)	10 (8.40)
Liver	1	0 (0.00)	0 (0.00)
Immune system			
Tonsil	22	13 (59.09)	32 (26.89)
MSLN*	12	4 (33.33)	10 (8.40)
TBLN*	11	1 (9.09)	1 (0.84)
Respiratory system			
Lung	49	7 (14.29)	14 (11.76)
Nasal swab	12	3 (25.00)	4 (3.36)
Total	**149**	**51 (34.23)**	**119 (100.00)**

* MSLN = Mesenteric Lymph node and TBLN = Tracheobronchial Lymph node.

4.2 Antimicrobial resistant and multidrug resistant for *Escherichia coli* isolates

A total of 119 *E. coli* isolates were tested for antimicrobial susceptibility of 13 antimicrobial agents from 9 different classes. *E. coli* isolates exhibited antimicrobial resistance to all classes except Fosfomycins. Detected *E. coli* isolates resisted to AMP 96.64% (115/119), TE 92.44% (110/119), SXT 81.51% (97/119), C 79.83% (95/119), NA 74.79% (89/119), CN 64.71% (77/119), CIP 62.18% (74/119), CTX 47.90% (57/119), CRO 42.86% (51/119), AMC 11.76% (14/119), CT 11.76% (14/119) and FOT 0.00% (0/119) respectively (Fig. 1). Furthermore, *E. coli* that resisted to AMP, C, NA, CN, CTX, and CRO were isolated from all organ samples. Dominant prevalence of antimicrobial resistant *E. coli* isolates in specific organ sample was not observed.

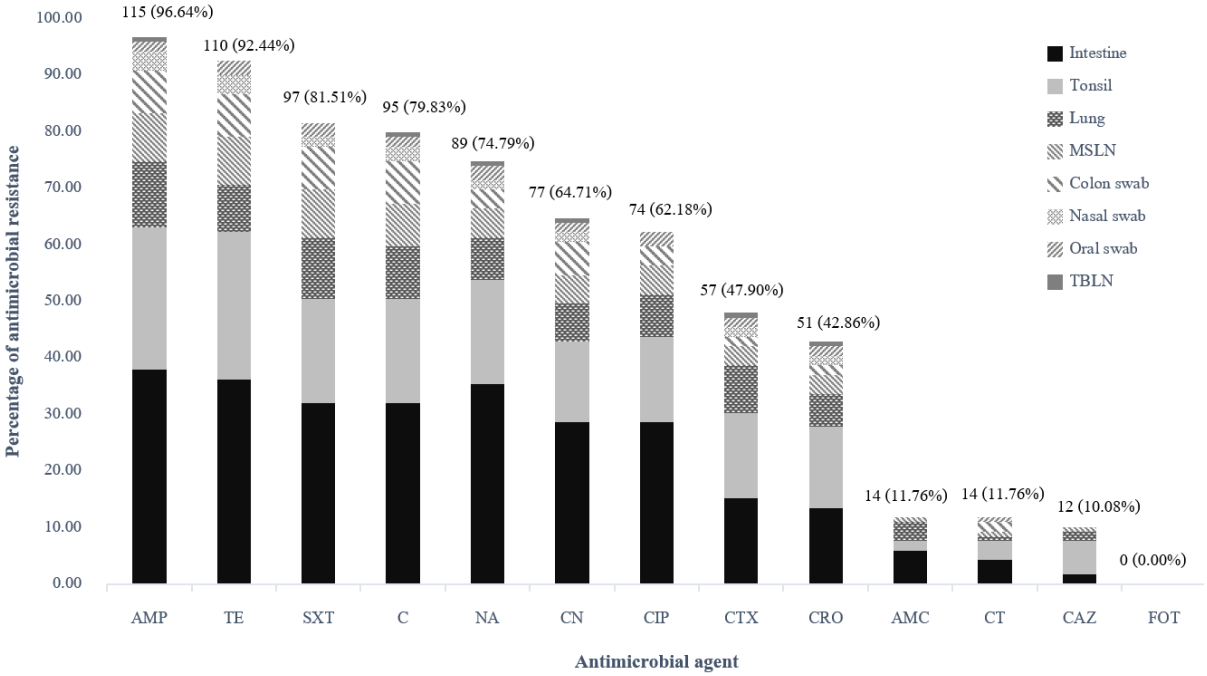

Fig. 1 Antimicrobial resistance among *Escherichia coli* isolated from diseased swine (N = 119). Abbreviations: Ampicillin (AMP), Tetracycline (TE), Trimethoprim/Sulfamethoxazole (SXT), Choramphenicol (C), Nalidixic acid (NA), Gentamicin (CN), Ciprofloxacin (CIP), Cefotaxime (CTX), Ceftriaxone (CRO), Amoxicillin/clavulanic acid (AMC), Colistin (CT), Ceftazidime (CAZ) and Fosfomycin (FOT).

The majority of *E. coli* isolates were MDR (94.96%; 113/119) (Table 2). The total 43 MDR patterns, excluding the AMP-TE and CN-TE, were determined. The total 2 most frequent MDR patterns were AMP-CRO-CTX-CIP-NA-C-CN-TE-SXT (12.61%; 15/119) and AMP-CIP-NA-C-CN-TE-SXT (12.61%; 15/119). Noteworthy, occurrence of other MDR patterns was obviously lower than these 2 patterns (Table 2). Furthermore, the *E. coli* possessing these 2 MDR patterns were isolated from various organ samples of swine in different farms (data not shown).

Table 2 Antimicrobial resistance patterns of *Escherichia coli* isolated from diseased swine (N = 119).

Antimicrobial resistance patterns	No. of antimicrobial agents	No. of antimicrobial class	Number of *E. coli* isolates (%)
AMP-TE	2	2	5 (4.20)
CN-TE	2	2	1 (0.84)
AMP-TE-SXT	3	3	3 (2.52)
AMP-C-SXT	3	3	1 (0.84)
AMP-C-TE-SXT	4	4	5 (4.20)
AMP-NA-C-TE	4	4	2 (1.68)
AMP-CTX-C-SXT	4	4	1 (0.84)
AMP-NA-TE-SXT	4	4	1 (0.84)
AMP-C-CN-SXT	4	4	1 (0.84)
AMP-CRO-CTX-C-SXT	5	4	4 (3.36)
AMP-CIP-NA-C-TE	5	4	2 (1.68)
AMP-C-CN-TE-SXT	5	5	2 (1.68)
AMP-CRO-CTX-CN-TE	5	4	1 (0.84)
AMP-CRO-CTX-TE-SXT	5	4	1 (0.84)
AMP-CIP-NA-TE-SXT	5	4	1 (0.84)
AMP-NA-CT-TE-SXT	5	5	1 (0.84)
AMP-CT-C-CN-SXT	5	5	1 (0.84)
AMP-CT-C-TE-SXT	5	5	1 (0.84)
AMP-CIP-NA-C-TE-SXT	6	5	8 (6.72)
CIP-NA-C-CN-TE-SXT	6	5	3 (2.52)
AMP-AMC-NA-CN-TE-SXT	6	5	2 (1.68)
AMP-CT-C-CN-TE-SXT	6	6	2 (1.68)
AMP-CRO-CTX-NA-C-CN	6	5	1 (0.84)
AMP-CIP-NA-C-CN-TE-SXT	7	6	15 (12.61)
AMP-AMC-CTX-NA-CN-TE-SXT	7	6	3 (2.52)
AMP-CRO-CTX-NA-C-CN-TE	7	6	3 (2.52)
AMP-CRO-CTX-CIP-NA-C-TE	7	5	1 (0.84)
AMP-CRO-CTX-C-CN-TE-SXT	7	6	1 (0.84)
AMP-CRO-CTX-NA-C-TE-SXT	7	6	1 (0.84)
AMP-CRO-CTX-CIP-NA-C-CN-TE	8	6	5 (4.20)
AMP-AMC-CTX-CIP-NA-CN-TE-SXT	8	6	2 (1.68)
AMP-AMC-CIP-NA-C-CN-TE-SXT	8	6	2 (1.68)
AMP-CIP-NA-CT-C-CN-TE-SXT	8	7	2 (1.68)
AMP-AMC-CIP-NA-CT-C-TE-SXT	8	6	1 (0.84)
AMP-CRO-CTX-CIP-NA-CT-TE-SXT	8	6	1 (0.84)
AMP-CRO-CTX-CIP-NA-C-TE-SXT	8	6	1 (0.84
AMP-CRO-CTX-NA-C-CN-TE-SXT	8	7	1 (0.84)
AMP-CRO-CTX-CIP-NA-C-CN-TE-SXT	9	7	15 (12.61)
AMP-CAZ-CRO-CTX-NA-CT-C-TE-SXT	9	7	1 (0.84)
AMP-CAZ-CRO-CTX-CIP-NA-C-CN-TE	9	6	1 (0.84)
AMP-CAZ-CRO-CTX-CIP-NA-C-CN-TE-SXT	10	7	5 (4.20)
AMP-CRO-CTX-CIP-NA-CT-C-CN-TE-SXT	10	8	3 (2.52)
AMP-CAZ-CRO-CTX-CIP-NA-CT-C-TE-SXT	10	7	1 (0.84)
AMP-AMC-CAZ-CRO-CTX-CIP-NA-CN-TE-SXT	10	6	2 (1.68)
AMP-AMC-CAZ-CRO-CTX-CIP-NA-C-CN-TE-SXT	11	7	2 (1.68)
Total			**119 (100.00)**

5. Discussion

E. coli is commonly found in human and mammal gastrointestinal tracts (Kaper, Nataro, & Mobley, 2004). The present research reported the prevalence of *E. coli* isolated from intestine and extraintestinal organ samples of diseased swine in Nakhon Pathom, Thailand. A previous study showed that the isolation of *E. coli* in serum and feces are associated with co-infection of porcine reproductive and respiratory syndrome virus (PRRSV) and porcine circovirus type 2 (PCV2) (Niederwerder et al., 2016). Due to lack of diagnostic record, association of swine disease and presence of extraintestinal *E. coli* in the present study were unable to be determined. Nevertheless, our study revealed remarkable detection of extraintestinal *E. coli* in tonsils (26.89%; 32/119) and lungs (11.76%; 14/119).

The present study reported that detected *E. coli* isolates resisted to AMP 96.64% (115/119), TE 92.44% (110/119), SXT 81.51% (97/119), C 79.83% (95/119), CN 64.71% (77/119) and CIP 62.18% (74/119) (Figure 1). These isolates were obtained from samples in local farms in Nakhon Pathom province. Provided information for antimicrobial agent use in these farms was limited. Lugsomya et al. (2017) showed strong evidence that the detection rate of resistant *E. coli* from the swine farms operating without routine use of antimicrobial agent was lower than that from farms using antimicrobial agents for therapeutic and prophylactic. The percentage of antimicrobial resistant *E. coli* from these 3 different farm types were AMP (97.0%, 98.6% and 100.0%), TE (83.6%, 88.6% and 100%), SXT (23.9%, 18.6% and 90.2%), C (29.9%, 25.7% and 100%) and CN (19.4%, 24.3% and 78.4%) resistance, respectively. A high detection rate of antimicrobial resistant *E. coli* in swine, particularly for AMP and TE, in previous and our studies indicate a substantial risk for public health caused by transmission antimicrobial resistant isolates from swine to human (Kirchner et al., 2014; Lugsomya et al., 2017).

In addition, MDR *E. coli* isolates were detected at 94.96% (113/119) from multiple organs of diseased swine in the present study. A recent study also reported relatively high MDR *E. coli* at 80.67% (242/300) from different parts of swine samples i.e. skin surfaces, fresh feces, and pork products from markets (Fang, Shen, Qu, and Han, 2019). Furthermore, in our study, the two most frequent MDR patterns including AMP-CRO-CTX-CIP-NA-C-CN-TE-SXT (12.61%; 15/113) and AMP-CIP-NA-C-CN-TE-SXT (12.61%; 15/113) were observed. Comparatively, these 2 MDR patterns are obviously higher than other patterns (Table 2). Since antimicrobial resistance of *E. coli* is strongly associated with a possession of antimicrobial resistance genes (Mazurek et al., 2018) and plasmid containing antimicrobial resistance gene cassette (Brilhante et al., 2019). The plasmid profiling of the isolates containing these 2 MDR patterns is suggested for further research to understand molecular evolution for antimicrobial resistance of isolated *E. coli* in diseased swine.

6. Conclusion

The present study reported antimicrobial resistance profiles of *E. coli* isolated from multiple organ samples of diseased swine in Nakhon Pathom province, Thailand. Antimicrobial resistant *E. coli* isolates were detected from all organ samples. The majority of isolated *E. coli* resisted to AMP, TE, SXT, C, NA, CN and CIP, respectively. Among 43 observed MDR patterns, two patterns represented distinctively higher than others. This study revealed remarkable detection of antimicrobial resistant *E. coli* in both intestinal tract and extra-intestinal organs of diseased swine.

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