

# Antimicrobial Susceptibility Patterns of *Escherichia coli* in Diarrheal Piglet Fecal Samples: Using Continuous Medicated Feed and Geographical Variation

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## Abstract

The aim of this study was to determine the minimal inhibitory concentrations (MIC) of antimicrobials against *Escherichia coli* by broth microdilution method and study antimicrobial sensitivity patterns for *E. coli* isolated from diarrheic piglets in pig farms that regularly use medicated feed. Total of 120 fecal swabs were collected from 3 conventional pig farms, located in Ratchaburi and Kanchanaburi provinces, for *E. coli* isolation. Only 114 samples (95.0%) were able to be isolated, 93.8% non-hemolytic *E. coli* and 6.2% hemolytic *E. coli*. Enrofloxacin showed the lowest MIC<sub>90</sub> (1 µg/ml), while the most susceptibility of *E. coli* was apramycin (100%). The sensitivity patterns of Farm 1 and 2 were not different, however, their sensitivity patterns were different from Farm 3 ( $P < 0.05$ ). The present result showed that there was no relationship between feed medication and *E. coli* antimicrobials resistance in piglets. In conclusion, apramycin and enrofloxacin were the most effective antimicrobial, considering sensitivity test in order to treat infection with *E. coli*. Whereas, lincomycin, and tylosin were the most resistance (100%).

**Keywords:** *Escherichia coli*, piglet, antimicrobial susceptibility, medicated feed, geography

# รูปแบบความไวต่อยาต้านจุลชีพของ *Escherichia coli* ในตัวอย่างมูลจาก ลูกสุกรท้องเสีย: การใช้ยาในอาหารเป็นประจำและความแตกต่างของพื้นที่

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## บทคัดย่อ

งานวิจัยนี้มีวัตถุประสงค์เพื่อศึกษารูปแบบความไวต่อยาต้านจุลชีพโดยประเมินค่าความเข้มข้นที่ต่ำที่สุดของยาที่สามารถยับยั้งการเจริญเติบโต (Minimal Inhibitory Concentration; MIC) ของเชื้อ *Escherichia coli* ที่แยกได้จากตัวอย่างอุจจาระของลูกสุกรท้องเสียในฟาร์มสุกรที่เลี้ยงด้านจุลชีพผสมอาหารเป็นประจำโดยวิธี broth microdilution สุ่มเก็บตัวอย่างจากอุจจาระลูกสุกรท้องเสียจำนวน 120 ตัวอย่าง จาก 3 ฟาร์ม ซึ่งเป็นโรงเรือนแบบเปิดและตั้งอยู่ในจังหวัดราชบุรีและกาญจนบุรี เพื่อนำมาแยกเชื้อ *E. coli* พบว่าเชื้อที่แยกได้เป็น *E. coli* 114 ตัวอย่าง (95.0%) โดยเป็น non hemolytic *E. coli* 93.8% และ hemolytic *E. coli* 6.2% ผลการศึกษาความไวต่อยาพบว่า ยาที่มีค่า MIC<sub>90</sub> ต่ำที่สุด (1 ไมโครกรัม/มิลลิลิตร) คือ เอนโรฟล็อกซาซิน ยาที่มีความไวต่อเชื้อ *E. coli* สูงที่สุดในทุกฟาร์ม คือ เพรอามัยซิน (100%) ส่วนยาที่ดื้อต่อเชื้อ *E. coli* คือ ไทโลซินและลินโคมัยซิน (100%) จากผลการศึกษายังพบว่าค่าความไวต่อยาของฟาร์ม 1 และ 2 ไม่มีความแตกต่างกัน แต่ทั้งสองฟาร์มนี้มีความแตกต่างอย่างมีนัยสำคัญเมื่อเทียบกับฟาร์ม 3 จากผลการศึกษาสรุปได้ว่าไม่มีความสัมพันธ์ระหว่างการใช้ยาผสมอาหารกับการดื้อยาของเชื้อ *E. coli* ในสุกร อย่างไรก็ตามฟาร์มควรมีการทดสอบหาค่าความไวยาของแต่ละฟาร์มเพื่อจะได้ค่าความไวต่อยาที่ถูกต้องและสามารถนำไปใช้ได้อย่างมีประสิทธิภาพมากที่สุด

คำสำคัญ: *Escherichia coli*, ลูกสุกร, ความไวต่อยาต้านจุลชีพ, อาหารผสมยา, พื้นที่

## Introduction

*Escherichia coli* infection in pigs causes diarrhea and may develop into economic losses for the pig production. *E. coli* is an important pathogen that can be divided by the nature of disease, for example, enterotoxigenic *E. coli* (ETEC), enteropathogenic *E. coli* (EPEC) and edema disease *E. coli* (EDEC) (Fairbrother and Gyles 2006). The disease is named based on the period of infected pigs, in suckling piglets called neonatal diarrhea, while in nursery pigs called post-weaning diarrhea (Choi and Chae 1999; Osek 1999; Fairbrother and Gyles 2006).

In the past few decades, the concept of using sub-therapeutic dose of antimicrobial agents as growth promoter has been accepted and applied in several pig farms in Thailand. However, the recommended sub-therapeutic dose is not enough to promote growth and health since it cannot protect pigs from bacterial infection, especially *E. coli*. Pig producers increase dosage until reach the full dose, however, the main purpose is changed from growth promotion to only prevention of bacterial infections. The use of medicated feed in pig industry contributed to the evolution and selection of resistant bacteria (Presscott 2004).

The studies of *E. coli* in Thailand showed the infection rate in piglets was ranged from 30% to 80% (Kortheerakul et al. 1987; Assavacheep et al. 2003). For antimicrobial susceptibility of *E. coli* in piglets, a study has been demonstrated that *E. coli* is sensitive to colistin, apramycin and gentamicin (Assavacheep et al. 2003). However, *E. coli* resistance to antimicrobials depends on various factors such as age of pigs, stressors and dosage (Mathew et al. 1998, 2003).

Medicated feed used in farm animals is often an issue in debate that causes the resistance of bacteria. In Thailand, pig producers use medicated feed as routinely but the resistance *E. coli* in human may cause from misuse of antimicrobials behavior. Therefore, it is important to

study antimicrobial susceptibility patterns and determine the minimal inhibitory concentration (MIC) of antimicrobials from diarrheic pigs in routine use medicated feed in pig farms in different geographical regions.

## Materials and Methods

Three pig farms, in Ratchaburi and Kanchanaburi provinces, were selected in order to collect fecal specimens from diarrheic pigs by rectal swab. Ten samples per month per farm (1 sample/pig), were collected for 4 months, from pre- and post-weaning piglets with diarrhea. Rectal swabs, in transport medium, were performed as aseptic technique. The samples were transferred to laboratory for culture on MacConkey and blood agars. The bacterial colonies were identified by biochemical tests such as gram's stain, methyl red, indole, Simmon citrate and triple sugar iron. Then, isolates were stored in 15% glycerol at  $-80^{\circ}\text{C}$  for further antimicrobial susceptibility testing.

Antimicrobial susceptibility tests were carried out on each isolates by broth microdilution method to determine MIC using Muller-Hinton broth according to the guidelines of the clinical and laboratory standards institute (CLSI) (2006). All dilutions were ranged from 0.03 to 64  $\mu\text{g/ml}$ . Ten antimicrobials, widely used in pig farms in Thailand, were chosen : apramycin, cephalixin, colistin, doxycycline, enrofloxacin, gentamicin, kanamycin, lincomycin, neomycin and tylosin. The MIC interpretative standards ( $\mu\text{g/ml}$ ) for *E. coli* were listed in Table 1. *E. coli* ATCC25922 was used in all tests for control potency of antimicrobial susceptibility assessment.

Results of MIC were analyzed by descriptive statistics. Among percentage of sensitivity was examined by Kruskal Wallis test. Comparison between percentages of sensitivity of antimicrobial used in this study was tested by Mann-Whitney U test. The statistical analysis was performed by SPSS statistics version 18 (SPSS Inc, IL) and  $P < 0.05$  was considered as statistical significance.

**Table 1** Antimicrobials used and MIC interpretation of 120 isolates *E. coli* from diarrhetic pigs

Antimicrobials	MIC breakpoint ( $\mu\text{g/ml}$ )*												
	Farm 1			Farm 2			Farm 3			All farms			
S	I	R	Min <sup>†</sup>	Max <sup>†</sup>	Mode <sup>†</sup>	Min	Max	Mode	Min	Max	Mode	MIC <sub>50</sub> <sup>‡</sup>	MIC <sub>90</sub> <sup>‡</sup>
Apramycin	< 16	-	> 16	1	4	4	2	4	2	4	4	4	4
Cephalixin	$\leq 8$	16	$\geq 32$	2	4	4	2	8	4	16	8	4	16
Colistin	$\leq 0.5$	1-2	$\geq 4$	1	4	4	1	4	2	16	8	2	8
Doxycycline	$\leq 4$	8	$\geq 16$	32	64	32	2	32	8	64	16	16	32
Enrofloxacin	$\leq 0.5$	-	$\geq 4$	0.03	0.06	0.03	0.03	0.06	0.06	32	0.25	0.06	1
Gentamicin	$\leq 4$	8	$\geq 16$	0.125	8	0.25	0.125	16	0.5	32	8	0.5	16
Kanamycin	$\leq 16$	32	$\geq 64$	2	4	4	2	4	2	64	64	4	64
Lincomycin	$\leq 0.5$	1-2	$\geq 4$	64	64	64	64	64	64	64	64	64	64
Neomycin	< 6	-	$\geq 25$	2	16	8	1	4	2	16	2	4	8
Tylosin	0.3-5	10	$\geq 20$	64	64	64	64	64	64	64	64	64	64

\* S: susceptible, I: intermediate, R: resistance

† Min: minimum, Max: maximum, Mode: the MIC value that occurs frequently.

‡ MIC<sub>50</sub> and MIC<sub>90</sub> are the concentration at 50% and 90% of the isolates of *E. coli* found susceptible. They are cumulative percentage of susceptibility.

## Results

All of 120 rectal swabs were collected from 3 pig farms. Altogether 114 out of 120 (95.0%) samples

could isolate *E. coli* which was non-hemolytic *E. coli* (93.8%) and hemolytic *E. coli* (6.2%) as shown in Table 2.

**Table 2** Results of *E. coli* isolation.

Farm	<i>E.coli</i>	Hemolytic <i>E.coli</i>	Non-hemolytic <i>E.coli</i>
1	100% (40/40)	2.5% (1/40)	97.5% (39/40)
2	92.5% (37/40)	2.7% (1/37)	97.3% (36/37)
3	92.5% (37/40)	13.5% (5/37)	86.5% (32/37)
	Mean ± SD	6.2 ± 6.3	93.8 ± 6.3

The minimum, maximum and mode of MICs of antimicrobials by farm and the cumulative percentage of susceptibility, MIC<sub>50</sub> and MIC<sub>90</sub>, were presented in Table 1. Mode is the most frequently occurring of MIC values. Enrofloxacin showed the lowest MIC<sub>90</sub> (1 µg/ml), followed by apramycin (4 µg/ml).

The extent sensitivity of *E. coli* strains differed between the sources of the isolates. *E. coli* isolates of Farm 1 were 100% sensitive to apramycin, kanamycin, cephalixin and enrofloxacin. The result of Farm 2 was similar with Farm 1, but plus one more antibiotic, neomycin. However, isolates from Farm 3 were only sensitive to apramycin. All isolates were resistant to tylosin and lincomycin. Farm 2 showed the highest percentage of susceptibility (77.5%) but not differ from Farm 1 (74.4%). Nevertheless, both farms were statistical significant from Farm 3 ( $P < 0.05$ ).

The median values of susceptibility test of all antimicrobials were presented in Table 3. Comparison among sensitive antimicrobials was significant difference ( $P < 0.01$ ) but not in intermediate and resistance. Tylosin, lincomycin and colistin were different in percentage of sensitivity from other antimicrobial agents.

## Discussion

The isolation results of this study are agreement with the study of Assavacheep et al. (2003) in that non-hemolytic *E. coli* is the major isolates. The results of MIC<sub>90</sub> revealed that enrofloxacin has the lowest MIC<sub>90</sub> when compare to other antimicrobial agents. This may be due to the pig farmers do not use enrofloxacin in feed for long time ago since its bitter taste. A study in Korea has been demonstrated that *E. coli* has low MIC<sub>90</sub> for ceftiofur and high MIC<sub>90</sub> in several drugs for instance, enrofloxacin, tylosin, lincomycin, gentamicin and tetracycline (Choi et al. 2002). The same situation was found in this study for tylosin and lincomycin, while enrofloxacin and gentamicin are practically susceptible.

The percentage of sensitivity, *E. coli* was 100% sensitive to apramycin in all farms when compare to enrofloxacin which was consistent with previous studies (Mathew et al. 1998; Assavacheep et al. 2003). The use of apramycin on the farm affects apramycin/gentamicin cross-resistant *E. coli* in pigs (Jensen et al. 2006). Nevertheless, in this study, Farm 3 showed the lowest sensitivity of gentamicin but apramycin still showed high sensitivity.

According to the previous report on sensitivity test, colistin is a drug of choice which pig producers often use to control *E. coli* infection. This study found

**Table 3** Median value of antimicrobial susceptibility test which presented in percentage.

	Antimicrobials*										P-value
	Apra	Cep	Tylo	Doxy	Neo	Kana	Enro	Linco	Colis	Genta	
%Sensitivity	100 <sup>a</sup>	95 <sup>ab</sup>	0 <sup>d</sup>	0 <sup>cd</sup>	50 <sup>bc</sup>	100 <sup>abc</sup>	100 <sup>ab</sup>	0 <sup>d</sup>	0 <sup>d</sup>	80 <sup>b</sup>	0.003 <sup>**</sup>
%Intermediate	0	0	0	12.5	42.5	0	0	0	55	10	NA
%Resistant	0	0	0	87.5	5	0	0	100	45	10	NA

\*Antimicrobials tested: Apra = Apramycin; Cep = Cephalixin; Tylo = Tylosin; Doxy = Doxycycline; Neo = Neomycin; Kana = Kanamycin; Enro = Enrofloxacin; Linco = Lincomycin; Colis = Colistin; Genta = Gentamicin

\*\*Comparison among percentage of susceptibility was performed by Kruskal-Wallis test

NA = Not applicable

<sup>a,b,c,d</sup>Comparison between antimicrobial. The significant difference ( $P < 0.05$ ) was presented as unlike superscript by Mann-Whitney U test

that *E. coli* from all farms showed resistant to colistin which is different from previously studied by Assavacheep et al. (2003). For the results of correlation, there is no correlation of antimicrobial used among 3 farms. Farm 3 did not use colistin in feed but found 100% resistance. Likewise, Farm 1 did not use colistin but found 55% intermediate and 45% resistance. In contrast, Farm 2 used colistin for prevention of *E. coli* infection, but found only 5% resistance and 95% intermediate.

All farms did not use tylosin, nevertheless, the results showed *E. coli* resist to tylosin (100%). Macrolides resistance can occur together with lincosamides resistance which known as macrolide-lincosamide-streptogramin B (MLSB) resistance (Roberts et al. 1999). Nowadays, there are new synthetic groups related to macrolide and add them to be a group of resistance, named MLSKO (Roberts 2008). This study found all isolates resist to tylosin as well as lincomycin which is same as a study in Korea (Choi et al. 2002).

Tetracycline group is frequently used in feed because of broad spectrum. Studies of Teshager et al. (2000) and Hsu et al. (2006) have been demonstrated that *E. coli* highly resists to several antimicrobial agents, including tetracycline. In this study, *E. coli* was resisted to doxycycline, a member of tetracycline group, 100% resistance in Farm 1 and 2, while only 35% resistance in Farm 3.

The location of farms may be a factor as farmers use similar medication program in the same area. Farm 1 and 2 are located in the same province but Farm 3 is in the different area. Overall antimicrobial susceptibility patterns in Farm 1 and 2 was quite similar, the sensitivity was 74.4% and 77.5%, respectively, while the sensitivity of Farm 3 was 35.0%. Therefore, the antimicrobial susceptibility patterns of *E. coli* may be different by geographical regions as previous report (Parveen et al. 2006).

Although only three farms were chosen, this study revealed that regular use of medicated feed for control bacterial infection may not induce resistance of

*E. coli* which is consistent with a study by Nijsten et al. (1996) reported that resistance patterns of *E. coli* between pigs and farmers are similar only 4%. The resistance of *E. coli* has variety of factors, not only medicated feed used in pig industry. The antibiotic usage behavior in human also affects the drug resistance. As a result, observations on the medication in pig industry influenced the antimicrobial resistance of *E. coli* in human may not be only the factor. Further study and continue monitoring program may help to answer this problem and the antimicrobial resistance in the field. However, this study shows that the use of medicated feed does not correlate to antimicrobial resistance of *E. coli* and it is different region-by-region.

In conclusion, this study shows that apramycin has the highest percentage of sensitivity and isolates were susceptible to enrofloxacin with the lowest MIC<sub>90</sub>. On the other hand, lincomycin and tylosin were the most resistance. Therefore, apramycin and enrofloxacin should be a drug of choice in order to treat *E. coli* infection in pig farms in Thailand. In addition, the sensitivity profile was different from farm-to-farm, each farms should perform antimicrobial susceptibility test for their own farm.

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