Veterinary Integrative Science 2019; 17(1): 11-20

Vet Integr Sci

Veterinary Integrative Sciences

ISSN; 2629-9968 (online)

Website; www.vet.cmu.ac.th/cmvj

Research article

Antimicrobial resistant profiles of *Escherichia coli* and contaminated *Salmonella* spp. from pork and butcher shops

Pramualchai Ketkhao[1,2], Sukanya Thongratsakul[3], Chaithep Poolkhet[3], Worawidh Wajjwalku[4] and Patamabhorn Amavisit[5,*]

[1]*Center for Agricultural Biotechnology, Kasetsart University Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*
[2]*Center of Excellence on Agricultural Biotechnology: (AG-BIO/PERDO-CHE), Bangkok 10900, Thailand*
[3]*Department of Veterinary Public Health, Faculty of Veterinary Medicine, Kasetsart University, Nakhon Pathom 73140, Thailand*
[4]*Faculty of Veterinary Medicine, Kasetsart University, Kamphaeng Saen Campus, Nakhon Pathom 73140, Thailand*
[5]*Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand*

Abstract

Antimicrobial resistant profiles of *Escherichia coli* isolated from pork and environment samples of six retail butcher shops in Bangkok, Thailand, were studied. Of the total samples, 73.3% were positive for *Salmonella* spp. and 86.7% were positive for *E. coli*. *E. coli* were tested for the minimum inhibitory concentrations against 12 antimicrobial agents that are commonly prescribed for infection in humans. The resistances to quinolones comprising of moxifloxacin and ciprofloxacin were 15.4% and 11.5%, respectively. The β-lactams resistance was observed at less frequent rates at 9.6% for cefotaxime, 3.8% for amoxicillin/clavulanic acid, 3.8% for cefoxitin, and 1.9% for ceftazidime, but all isolates were sensitive to cefoperazone/sulbactam and cefpirome. Furthermore, 11.5% of the isolates produced ESBL enzymes. Aminoglycosides resistance was observed for gentamycin at 17.3% while amikacin resistance (0%) was not found. Trimethoprim/sulfamethoxazole showed the highest resistance at 57.7%. Only one colistin resistant isolate (MIC at 8 µg/ml) was found, but it did not carry the plasmid mediated colistin resistance genes *mcr-1* and *mcr-2*. Amplifying the gene integrase 1 (*intI1*), 3.9% of the isolates yielded positive PCRs. In this study, the antimicrobial resistant rates of *E. coli* from pork and markets were relatively low and *mcr* genes were not yet distributed in the tested *E. coli*.

Keywords: Antimicrobial resistance, *mcr*, MIC, Pork

***Corresponding author:** Patamabhorn Amavisit. Department of Microbiology and Immunology, Faculty of Veterinary Medicine, Kasetsart University, Bangkok 10900, Thailand. Email: fvetpaa@ku.ac.th

Article history: *received manuscript: 23 July 2018,*
revised manuscript: 23 August 2018,
accepted manuscript: 4 September 2018,
published online: 3 October 2018
Academic editor: *Korakot Nganvongpanit*

INTRODUCTION

The increase of antimicrobial resistant bacteria (AMR) infections has led to a rise of morbidity and mortality in humans. The World Health Organization has concluded that the adverse human health consequences were due to resistant organisms from both human and non-human usages of antimicrobials (WHO, 2003). Overuse of antimicrobials in animals could lead to the increase of AMR and contamination of AMR bacteria in meat which could directly contaminate consumers. However, the route of bacterial contamination in meat can occur not only in live animals but also in slaughterhouses, during meat processing, transportation, and butcher shops.

In Thailand, pork can be purchased from supermarkets and fresh markets. Supermarkets are air-conditioned, usually control the environment temperature and seem to have higher hygienic conditions than fresh markets. Fresh markets are open air, the foodstuffs are often open to the environment prior to sale and stored at ambient temperatures. Multiple sources of food contamination including rodents, insects, and sewage are regularly found in fresh markets (Vindigni et al., 2007).

Swine farms in Thailand have different management systems and sizes. Both large integrated farms, with fully vertical integrated systems, and small size farms in the backyard exist in Thailand (Thanapongtharm et al., 2016). On swine farms, colistin metaphylaxis, prophylaxis and treatments have been effectively used for the past two decades. Recently Thailand prohibited the prophylaxis use of colistin for swine, (DLD, 2017) because it is regarded as a last resort antimicrobial therapeutic option against carbapenem-resistant bacteria in humans. A rapid spread of colistin resistant bacteria has been reported in many countries (Kempf et al., 2016). Plasmid mediated colistin resistance genes, *mcr-1* to *mcr-5*, were discovered to be a rapid cause of resistance distribution (Liu et al., 2016; Xavier et al., 2016; Borowiak et al., 2017; Carattoli et al., 2017; Yin et al., 2017)

The aims of the present study were to determine AMR profiles and minimum inhibitory concentrations (MIC) of antimicrobial agents in *E. coli* isolated from pork and environment samples from butcher shops located in fresh markets. *E. coli* and *Salmonella* spp. contamination were investigated. The distribution of antimicrobial resistant genes, *mcr-1*, *mcr-2* and integrase (*intI1*) gene were examined by using PCR.

MATERIALS and METHODS

Sample collection and interview

The total of 60 samples including red meat (7), belly pork (5), ground pork (6), offal (14) and environment samples of cutting boards (6), weighing machines (6), tables (6), wastewater (6), knives (2) and pork grinders (2) were cross-sectionally collected from six retail butcher shops in Bangkok, Thailand from October to December 2016. The meat samples were kept in sterile plastic sampling bags and chilled in an ice box while environment samples were collected using sterile cotton swabs and placed in Amies transport medium (Oxoid, U.S.A.). Information about the pork handling, desk and floor cleaning program were collected from each shop vendor by using face-to-face inter-

view. The hygiene practices of the vendor were observed and the data were recorded.

Bacterial isolation

All the samples were cultured for *E. coli* and *Salmonella* spp. following the methods described by the International Organization for Standardization (ISO 9308–1, 2014 and ISO 6579, 2007, respectively). Three isolates of *E. coli* and three isolates of *Salmonella* spp. of each positive sample were kept in skim milk at -20°C for further study.

Serogroup test of *Salmonella* spp.

Agglutination tests on the basis of somatic O antigen, and phase 1 and phase 2 flagella antigens according to the White-Kauffaman-Le Minor scheme (Grimont and Weill, 2007) were performed. Antisera OMA and OMB (S & A Reagents Lab, Thailand) were used for testing *Salmonella* serogroup A, B, D, E, and C respectively.

Antimicrobial susceptibility test of *E. coli*

The *E. coli* isolates were tested for MIC against 12 antimicrobial agents, comprising of amoxicillin/clavulanic acid, ceftazidime, cefoperazone/sulbactam, cefpirome, cefotaxime, cefoxitin, amikacin, gentamicin, ciprofloxacin, moxifloxacin, colistin, and trimethoprim/sulfamethoxazole, and extended-spectrum β-lactamase (ESBL) was screened, by using a VITEK 2 compact automated machine (bioMérieux, Marcy-I'Etoile, France). ESBL confirmation test of VITEK 2 is based on simultaneous assessment in the inhibitory effects of cefepime (0.5 mg/L), cefotaxime (0.5 mg/L) and ceftazidime (1.0 mg/L) alone and in the combination with clavulanic acid (4, 4 and 10 mg/L, respectively). The reduction of growth within wells containing clavulanic acid and those which do not contain clavulanic acid indicates expression of an ESBL (Spanu et al., 2006). *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the quality control strains. Antimicrobial resistance breakpoints were interpreted following the Clinical and Laboratory Standards Institute (CLSI, 2014) criteria, except the cefpirome and moxifloxacin breakpoints were applied in accordance with Vitek 2 system software version 7.01 (bioMérieux, Marcy-I'Etoile, France)

Detection of plasmid mediated colistin resistance gene and integrase gene

E. coli DNA was prepared by using the boiling method. Each PCR reaction was 5X Phusion HF buffer (7.5 mM $MgCl_2$), 200 µM of each dNTP, and 0.5 µM of each forward and reverse primer, 0.02 units of Phusion Hot Start II High–Fidelity DNA polymerase (Thermo Scientific, USA) and 1 µl of the DNA template. The *mcr-1* and *mcr-2* genes were amplified by using primers CLR-F (5'-CGGTCAGTCCGTTTGTTC-'3), CLR-R (5'-CTTGGTCGGTCTGTAGGG-'3) and MCR2-IF (5'-TGTTGCTTGTGCCGATTGGA), MCR2-IR (5'-AGATGGTATTGTTGGTTGCTG-'3) following the methods previously described by Liu et al. (2016) and Xavier et al. (2016). Primers intI1-F (5'-GCATCCTCGGTTTTCTGG-'3) and intI1-R (5'-GGTGTGGCGGGGTTCGTG-'3) were used for amplifying the integrase gene, *intI* (Shibata

et al., 2003). The PCR conditions used for amplification were an initial denaturation step at 98 °C for 3 min; 35 cycles at 98 °C for 30 s, annealing 30 s at 55 °C for *mcr-1* and *mcr-2* or at 58 °C for *intI1*, then an extension at 72 °C for 35 s, followed by a final extension step at 72 °C for 5 min.

Data analysis

Descriptive statistics were used to describe the percentage and frequency of antimicrobial susceptibility, gene detection, the source of samples and interview data. Pearson's Chi-square test was used to analyze the relationship of *Salmonella* serogroups and sources of the samples by using NCSS 11 software (NCSS, Kaysville, UT). $P<0.05$ was considered statistically significant for the statistical tests.

RESULTS

Of the total samples collected from the six retail butcher shops, 86.7% (52/60) and 73.3% (44/60) were positive for *E. coli* and *Salmonella* spp., respectively. The frequencies of *E. coli* found in the pork samples of each butcher's shop were similar, between 80% and 100% (Table 1). A difference was found in the environmental samples of shop E where *E. coli* was not found to be present in the environmental samples and *Salmonella* spp was present in only one sample (cutting board).

The total *Salmonella* spp. isolates from the 44 samples presented three different frequencies of serogroup comprising of serogroup B at 40.9%, serogroup C at 50% and serogroup E at 9.1%. There were no significant differences in the serogroups of the pork and the environment samples ($P > 0.05$). Only four isolates (9.1%) were identified as serogroup E which were the samples isolated from shop D (n=3) and shop F (n=1).

Table 1 *E. coli* (n=52) isolated from retail pork and environment samples (n=60) of 6 butcher shops (A–F)

Sources	Number of *E. coli* positive samples (%)						
	A	B	C	D	E	F	Total
Pork	5/5 (100)	4/5 (80)	5/5 (100)	6/6 (100)	6/6 (100)	5/6 (83.3)	**31/33 (93.9)**
Environment	4/4 (100)	4/4 (100)	4/4 (100)	4/5 (80)	0/5 (0)	5/5 (100)	**21/27 (77.8)**
Total	**9/9 (100)**	**8/9 (88.9)**	**9/9 (100)**	**10/11 (90.9)**	**6/11 (54.5)**	**10/11 (90.9)**	**10/11 (90.9)**

Of the 52 isolates that were studied for MIC, five isolates were resistant to cefotaxime and only one isolate was resistant to ceftazidime (Table 2). All isolates were sensitive to cefoperazone/sulbactam and cefpirome. Six isolates (11.5%) of *E. coli* produced extended-spectrum β-lactamase enzymes. Of the six ESBL produced isolates, five were resistant to cefotaxime. The resistance to quinolone agents had similar rates which were 15.4% moxifloxacin and 11.5% ciprofloxacin. Aminoglycosides resistances were observed for gentamycin at 17.3% but amikacin resistance (0%) was not found. Of the 30 isolates that were resistant to trimethoprim/sulfamethoxazole, 17 isolates were single resistant to the antimicrobial only.

Table 2 Distribution of minimum inhibitory concentration (MIC) and antimicrobial resistance rates of *E. coli* (n=52) from pork and environment samples.

Agents[1]	MIC values (µg/ml)				Resistance (%)
	Breakpoints[2]	Range	MIC_{50}	MIC_{90}	
AMC	≥ 32	≤ 2– ≥ 32	4	8	3.8
CAZ	≥ 16	≤ 1– 16	≤ 1	≤ 1	1.9
CFP	≥ 64	≤ 8– 16	≤ 8	≤ 8	0
CPO[3]	≥ 64	≤ 1– 16	≤ 1	≤ 1	0
CTX	≥ 4	≤ 1– ≥ 64	≤ 1	≤ 1	9.6
CX	≥ 32	≤ 4– ≥ 64	≤ 4	16	3.8
AK	≥ 64	≤ 2– 4	≤ 2	4	0
GN	≥ 16	≤ 1– ≥ 16	≤ 1	≥ 16	17.3
CIP	≥ 4	≤ 0.25 – ≥ 4	≤ 0.25	4	11.5
MXF[3]	≥ 8	≤ 0.25 – ≥ 8	≤ 0.25	≥ 8	15.4
CL	≥ 8	≤ 0.50 – 8	≤ 0.50	≤ 0.50	1.9
SXT	≥ 80	≤ 20– ≥ 320	≥ 320	≥ 320	57.7

[1]AMC=amoxicillin/clavulanic acid, CAZ=ceftazidime, CFP=cefoperazone/sulbactam, CPO=cefpirome, CTX=cefotaxime, CX=cefoxitin, AK=amikacin, GN=gentamicin, CIP=ciprofloxacin, MXF=moxifloxacin, CL=colistin, SXT=trimethoprim/sulfamethoxazole. [2]antimicrobial breakpoints followed CLSI (2014) and [3]VITEK 2 automated machine (bioMérieux, Marcy-I'Etoile, France)

The isolates (n=14) that were resistant to ≥ 2 antimicrobial agents, presented 10 resistant patterns (Table 3). Regarding the criteria of multidrug resistant isolates "acquired non–susceptibility to at least one agent in three or more antimicrobial categories" (Magiorakos et al., 2012), there were only 7 (13.5%) multidrug resistant (MDR) *E. coli* comprising of five isolates from the pork and two isolates from the environment samples. These MDR isolates were found in three butcher shops (shop A, B, and D). Among MDR *E. coli*, five isolates also produced ESBL enzymes.

Identical resistant patterns were found in some particular isolates (Table 3). In shop A, the same pattern of CIP-MXF-SXT was found from the isolates of pork and the environmental swab from the weighing machine. In shop B, three MDR resistant patterns (6 agents) of the isolates from the ground pork (CAZ-CTX-GN-CIP-MXF-SXT), the table (CX-CTX-GN-CIP-MXF-SXT) and the pork (AMC-CX-GN-CIP-MXF-SXT) presented similar R-types.

Focusing on colistin resistant profile, only one *E. coli* from pork in shop D was resistant to colistin with the MIC at 8 µg/ml. This isolate had the MDR pattern of CIP-MXF-CL-SXT. However, the isolate yielded negative results for *mcr-1* and *mcr-2* plasmid mediated colistin resistant genes by PCR. Furthermore, the isolates were screened for the integrase gene (*intI1*) of class 1 integron by using DNA amplification. Only two isolates, from the weighing machine at shop B and the pork from shop D, yielded positive. The resistant patterns of these isolates were CTX-GN-SXT and CIP-MXF-CL-SXT, respectively.

Table 3 Antimicrobial resistance patterns of 31 resistant *E. coli* from pork and environment samples.

Number of agents	Resistance patterns[1]	Sources of *E. coli*			
		Pork (n=16)		Environment (n=15)	
		Shop	Frequency	Shop	Frequency
1 (n=17)	SXT			A	2
		B	1		
				C	3
		D	3	D	2
		E	2		
		F	1	F	3
2 (n=5)	AMC-SXT (ESBL)			D	1
	GN-SXT			D	1
	MXF-SXT	A	3		
3 (n=5)	CIP-MXF-SXT	A	1	A	1
	CTX-GN-MXF (ESBL)	A	1		
	CTX-GN-SXT (ESBL)	D	1	B	1
4 (n=1)	CIP-MXF-CL-SXT	D	1		
6 (n = 3)	AMC-CX-GN-CIP-MXF-SXT	B	1		
	CAZ-CTX-GN-CIP-MXF-SXT (ESBL)	B	1		
	CTX-CX-GN-CIP-MXF-SXT (ESBL)			B	1

[1]AMC=amoxicillin/clavulanic acid, CAZ=ceftazidime, CIP=ciprofloxacin, CL=colistin, CPO=cefpirome, CTX=cefotaxime, CX=cefoxitin, GN=gentamicin, MXF=moxifloxacin, SXT=trimethoprim/sulfamethoxazole, ESBL=extended-spectrum β-lactamases

DISCUSSION

Pork samples from the six retail butcher shops presented similar frequencies of *E. coli* and *Salmonella* while a difference was found in the environmental samples of shop E. By observation and interview at every shop, the pork was transported by mini-trucks that had temperature control and the meat was kept in refrigerators or ice containers at the markets. Shops A, B, C, D, and F obtained their meat from a local slaughterhouse near Bangkok, but shop E received its meat from standard farms that had quality control for every step of the production process. Every shop displayed the meat on the tables that were in an open area, but shop E displayed meat in a glass cabinet for pest prevention. However, the sources of the meat and the display condition were not related to the frequencies of the isolates in the pork.

The interview found that the tables and equipment of all the shops were routinely daily cleaned by using dishwashing liquid (linear alkyl benzene sulfonate, sodium salt, and sodium lauryl ether sulfate) and laundry detergent.

Sodium hydroxide was used to clean the market floors at shops A, B, C, and D, 1–2 times per week. Polyhexamethylene biguanide hydrochloride (PHMB) and alkyl dimethyl benzyl ammonium chloride (ADBAC) were utilized to clean the market floor at shop E and F every three months. In our study, the isolation rates of *E. coli* and *Salmonella* from the environmental samples of shop E were lower than the other shops at 54.6% and 36.4% respectively. However, the relationship between different disinfectants and bacterial contamination was not detected. By observation, the vendor of shop E wore disposable gloves during picked up the meat while the vendors of other shops did not. The lowest contamination of bacteria was demonstrated in shop E, this probably resulted from the better hygiene practices of the vendor.

The common *Salmonella* serogroup C and B found in this study were similar to other studies of pork in Thailand (Sanguankiat et al., 2010; Sinwat et al. 2016). *Salmonella* Rissen of serogroup C and *S*. Typhimurium of serogroup B have been reported to be predominantly in pork samples (n=69) in Thailand (Sinwat et al. 2016). Serogroup E comprising of common serovars including Welteredens, Anatum, etc. are found in the public health system in Thailand (Bangtrakulnonth et al., 2004), but were not found as frequently in this study.

The twelve antimicrobials in this study comprised of eight antimicrobials (amoxicillin/clavulanic acid, cefotaxime, ceftazidime, cefpirome, gentamicin, ciprofloxacin, moxifloxacin, and colistin) that were categorized into critically important antimicrobial by the WHO, 2017. These agents were used to treat various bacterial infections including severe or life-threatening issues in humans. The resistances to β-lactams including amoxicillin/clavulanic acid, ceftazidime, cefotaxime (3rd generation cephalosporin), and cefoxitin (2nd generation cephalosporin) in this study were relatively low. Of the six *E. coli* that produced ESBL, five isolates were resistant to cefotaxime but only one isolate was resistant to ceftazidime. There are a variety of ESBL enzymes that were encoded by different genes. One of the cefotaxime resistance enzyme is CTX-M-type β-lactamases (cefotaximase) which can be hydrolyzed by this antimicrobial particularly (Shaikh et al., 2015).

Resistance rates of quinolones including ciprofloxacin and moxifloxacin were slightly low. All of the ciprofloxacin resistant *E. coli* (n=6) were resistant to moxifloxacin. Moxifloxacin is a fourth generation fluoroquinolone with expanded activity against gram-positive and anaerobic organisms. However, these two agents have the same action mechanism for inhibition of type II DNA topoisomerases (gyrases) (Nightingale, 2000). Therefore, their susceptibility rates for *E. coli* are similar.

In this study, fortunately, *mcr-1* and *mcr-2* genes were not yet distributed in the tested *E. coli*. Colistin resistant *E. coli* from the pork presented at very low rates. This was similar to the former studies (Liu et al., 2016) that found low colistin resistant rates in pork but higher in pigs. Furthermore, the resistances of high generation antimicrobials that are used in human treatment presented significantly low among the isolates from the pork.

Using *E. coli* as the sentinel bacteria, their AMR profiles implied that cross contamination of resistant *E. coli* within the markets or transportation was probable but was improbable that any cross contamination emanated from swine farms. *E. coli* R types from swine farms were conducted by the same

researcher group and found understandable differences (data not shown). However, this hypothesis should be confirmed by molecular subtyping of these resistant isolates.

ACKNOWLEDGEMENTS

This study was supported by Center for Agricultural Biotechnology and the Center of Excellence on Agricultural Biotechnology (AG-BIO/PERDO-CHE), Kasetsart University and Thailand One Health University Network (THOHUN) (Subaward Agreement PO No: P004721405). Supaporn Juangphanich, Apasara Worarach, and Srisamai Viriyarumpa were appreciated for technical support

REFERENCES

Bangtrakulnonth, A., Pornreongwong, S., Pulsrikarn, C., Sawanpanyalert, P., Hendriksen, R.S., Lo Fo Wong, D.M.A., Aarestrup, F.M. 2004. *Salmonella* serovars from humans and other sources in Thailand, 1993-2002. Emerg. Infect. Dis. 10, 131–136.

Borowiak, M., Fischer, J., Hammerl, J.A., Hendriksen, R.S., Szabo, I., Malorny, B. 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar *Paratyphi B*. J. Antimicrob. Chemother. 72, 3317–3324.

Carattoli, A., Villa, L., Feudi, C., Curcio, L., Orsini, S., Luppi, A., Pezzotti, G., Magistrali, C.F. 2017. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro. Surveill. 22, 30589.

Clinical and Laboratory Standards Institute (CLSI). 2014. Performance standards for antimicrobial susceptibility testing; 24th Informational Supplement. M100-S24. Clinical and Laboratory Standards Institute. Wayne.

Department of Livestock Development (DLD). 2017. Strict controls on the use of colistin on farms. 8 February 2017. (In Thai)

Grimont, P.A.D., and Weill, F.X. 2007. Antigenic formulae of the *Salmonella* serovars, (9th ed.). World Health Organization Collaborating Center for Reference and Research on *Salmonella*, Institute Pasteur, Paris.

International Organization for Standardization. 2014. ISO 9308-1:2014. Water quality -- Enumeration of *Escherichia coli* and coliform bacteria -- Part 1: Membrane filtration method for waters with low bacterial background flora. International Organization for Standardization. Geneva.

International Organization for Standardization. 2007. ISO 6579:2007. Microbiology of food and animal feeding stuffs -- Horizontal method for the detection of *Salmonella* spp. International Organization for Standardization. Geneva.

Kempf, I., Jouy, E., Chauvin, C. 2016. Colistin use and colistin resistance in bacteria from animals. Int. J. Antimicrob. Agents. 48, 598–606.

Liu, Y.Y., Wang, Y., Walsh, T.R., Yi, L.X., Zhang, R., Spencer, J., Doi, Y., Tian, G., Dong, B., Huang, X., Yu, L.-F., Gu, D., Ren, H., Chen, X., Lv, L., He, D., Zhou, H., Liang, Z., Liu, J.H., Shen, J. 2016. Emergence of plasmid-mediated colistin resistance mechanism *MCR-1* in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect. Dis. 16, 161–168.

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281.

Nightingale, C.H. 2000. Moxifloxacin, a new antibiotic designed to treat community-acquired respiratory tract infections: a review of microbiologic and pharmacokinetic-pharmaco dynamic characteristics. Pharmacotherapy. 20, 245–256.

Sanguankiat, A., Pinthong, R., Padungtod, P., Baumann, M., Zessin, K.H., Srikitjakarn, L., Fries, R. 2010. A cross-sectional study of *Salmonella* in pork products in Chiang Mai, Thailand. Foodborne Pathog. Dis. 7, 873–878.

Shaikh, S., Fatima, J., Shakil, S., Rizvi, S.M.D., Kamal, M.A. 2015. Antibiotic resistance and extended spectrum beta-lactamases: Types, epidemiology and treatment. Saudi J. Biol. Sci. 22, 90–101.

Shibata, N., Doi, Y., Yamane, K., Yagi, T., Kurokawa, H., Shibayama, K., Kato, H., Kai, K., Arakawa, Y. 2003. PCR typing of genetic determinants for metallo-beta-lactamases and integrases carried by gram-negative bacteria isolated in Japan, with focus on the class 3 integron. J. Clin. Microbiol. 41, 5407–5413.

Spanu, T., Sanguinetti, M., Tumbarello, M., D'Inzeo, T., Fiori, B., Posteraro, B., Santangelo, R., Cauda, R., Fadda, G. 2006. Evaluation of the new VITEK 2 extended-spectrum beta-lactamase (ESBL) test for rapid detection of ESBL production in Enterobacteriaceae isolates. J. Clin. Microbiol. 44, 3257–3262.

Sinwat, N., Angkittitrakul, S., Coulson, K.F., Pilapil, F.M.I.R., Meunsene, D., Chuanchuen, R. 2016. High prevalence and molecular characteristics of multidrug-resistant *Salmonella* in pigs, pork and humans in Thailand and Laos provinces. J. Med. Microbiol. 65, 1182–1193.

Thanapongtharm, W., Linard, C., Chinson, P., Kasemsuwan, S., Visser, M., Gaughan, A.E., Epprech, M., Robinson, T.P., Gilbert, M. 2016. Spatial analysis and characteristics of pig farming in Thailand. BMC Vet. Res. 12, 218.

Vindigni, S.M., Srijan, A., Wongstitwilairoong, B., Marcus, R., Meek, J., Riley, P.L., Mason, C. 2007. Prevalence of foodborne microorganisms in retail foods in Thailand. Foodborne Pathog. Dis. 4, 208–215.

World Health Organization (WHO). 2003. Joint FAO/OIE/WHO expert workshop on non-human antimicrobial usage and antimicrobial resistance: scientific assessment. [Online] Available: http://apps.who.int/iris/bitstream/10665/68883/1/WHO_CDS_CPE_ZFK_2004.7.pdf.

World Health Organization (WHO). 2017. Critically important antimicrobials for human medicine, 5th revision 2016 World Health Organization. [Online] Available: http://www.who.int/foodsafety/publications/antimicrobials-fifth/en/.

Xavier, B.B., Lammens, C., Ruhal, R., Kumar-Singh, S., Butaye, P., Goossens, H., Malhotra-Kumar, S. 2016. Identification of a novel plasmid-mediated colistin-resistance gene, *mcr-2*, in *Escherichia coli*, Belgium, June 2016. Euro. Surveill. 21. doi: 10.2807/1560-7917.ES.2016.21.27.30280.

Yin, W., Li, H., Shen, Y., Liu, Z., Wang, S., Shen, Z., Zhang, R., Walsh, T.R., Shen, J., Wang, Y. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. MBio. 8, e00543-17. doi: 10.1128/mBio.00543-17.

How to cite this article;

Pramualchai Ketkhao, Sukanya Thongratsakul, Chaithep Poolkhet, Worawidh Wajjwalku and Patamabhorn Amavisit. Antimicrobial resistant profiles of *Escherichia coli* and contaminated *Salmonella* spp. from pork and butcher shops. Veterinary Integrative Sciences. 2019; 17(1): 11-20