The Thai Journal of Veterinary Medicine

Volume 42
Issue 2 *July, 2012*

Article 15

7-1-2012

Plasmid Profiles of Multidrug-resistant Escherichia coli from Clinically Healthy Swine

Khin Khin Lay

Nisit Chansong

Rungtip Chuanchuen

Recommended Citation

Khin Lay, Khin; Chansong, Nisit; and Chuanchuen, Rungtip (2012) "Plasmid Profiles of Multidrug-resistant Escherichia coli from Clinically Healthy Swine," *The Thai Journal of Veterinary Medicine*: Vol. 42: Iss. 2, Article 15.
DOI: https://doi.org/10.56808/2985-1130.2388
Available at: https://digital.car.chula.ac.th/tjvm/vol42/iss2/15

Short Communication

Plasmid Profiles of Multidrug-resistant *Escherichia coli* from Clinically Healthy Swine

Khin Khin Lay[1] Nisit Chansong[2] Rungtip Chuanchuen[1]*

Abstract

A total of 145 *Escherichia coli* isolates originated from clinically-healthy pigs were analyzed for plasmid profiles. All but one isolate (*n*=144) carried one to nine plasmids with size ranging from 0.7-16.2 Mda. Fifty-six plasmid profiles were defined based on sizes and numbers of plasmids in each strain, of which the most common patterns were 15.8, 4, 3.3, 1.7, 1.3 Mda and 16.2, 15.8, 4, 3.3, 2.3, 1.7, 1.3, 1, 0.7 Mda. The results indicate wide distribution of plasmids among the commensal *E. coli* isolates originated from clinically-healthy pigs.

Keywords: *Escherichia coli*, plasmid profile, swine

[1]*Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University, Bangkok 10330*
[2]*Novatis (Thailand) Ltd., Bangkok 10110 Thailand*
Corresponding author E-mail: *rchuanchuen@yahoo.com*

บทคัดย่อ

รูปแบบพลาสมิดของ *Escherichia coli* ดื้อยาหลายชนิดพร้อมกันที่แยกได้จากสุกรที่มีสุขภาพดี

คิน คิน เล[1] นิศิษฐ จันทร์ทรง[2] รุ่งทิพย์ ชวนชื่น[1]

ศึกษารูปแบบพลาสมิดใน *Escherichia coli* จำนวน 145 isolates จากสุกรที่มีสุขภาพดี พบว่าเชื้อจำนวน 144 isolates มีพลาสมิดขนาด 0.7-16.2 Mda นำมาจัดรูปแบบพลาสมิดตามขนาดและจำนวนของพลาสมิดได้ 56 รูปแบบ โดยรูปแบบที่พบมากที่สุด คือ 15.8, 4, 3.3, 1.7, 1.3 Mda และ 16.2, 15.8, 4, 3.3, 2.3, 1.7, 1.3, 1, 0.7 Mda. ผลการศึกษาชี้ให้เห็นว่ามีการกระจายตัวอย่างกว้างขวางของพลาสมิดใน *E. coli* ที่เป็นแบคทีเรียประจำถิ่น

คำสำคัญ: เอสเชอริเคีย คอลัย, รูปแบบพลาสมิด, สุกร

[1]*ภาควิชาสัตวแพทยสาธารณสุข คณะสัตวแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย กรุงเทพฯ 10330*

[2]*บริษัท โนวาทีส (ประเทศไทย) จำกัด กรุงเทพฯ 10110 ประเทศไทย*

***ผู้รับผิดชอบบทความ E-mail:** rchuanchuen@yahoo.com*

Introduction

Pigs serve as an important source of food for world communities and also play a role as a major reservoir for many species of bacteria. Such bacterial species include both pathogenic and non-pathogenic strains, of which *Escherichia coli* is prevalent in intestinal tracts of pigs. Most *E. coli* serotypes are harmless and considered normal flora that could yield benefits to the hosts. However, some strains inflict diseases in humans and animals. Evidently, commensal *E. coli* act as a reservoir for transmission of numerous antimicrobial resistance-encoding determinants. Since clinically-healthy pigs are closed to markets and food chain, *E. coli* in healthy pigs can pose a risk to consumers and contributes to cross-contamination among carcasses in slaughtering plants.

A plasmid is an extra-chromosomal DNA molecule that has a key role in the horizontal transfer of genetic information between bacteria. Several antimicrobial resistant genes are plasmid mediated. In particular, class 1 integrons that are mobile elements have been shown to be located on conjugative plasmids (Hsu et al., 2006). Class 1 integrons compose of two conserved segments (CS), 5′-CS containing an integrase gene (*intI1*) and 3′-CS typically containing *qacEΔ1* (encoding resistance to quaternary ammonium compounds), *sul1* (encoding resistance to sulphonamide). These two regions are separated by a variable region that contains one or more gene cassettes. Due to the possession of several gene cassettes, class 1 integrons contribute to multi-resistance in bacteria (Fluit and Schmitz, 2004). Horizontal transfer of bacterial plasmid is one of the most efficient routes of resistant gene spread and, therefore, principally contributed to wide dissemination of multidrug resistant traits among *E. coli* and other bacterial species (Dobrindt et al., 2002; Maynard et al., 2004; Moreira et al., 2005). In this matter, antimicrobial use in pig production is the main reason for persistence and circulation of resistance plasmids in bacteria.

Minimizing plasmid transfer is crucial for control of spreads of multidrug-resistant *E. coli*. In this case, understanding of molecular epidemiology of resistant plasmids is firstly required. Several studies previously described characteristics of plasmids in *E. coli* originated from humans and animals. However, analysis of plasmids is unceasingly needed to update and trace evolutionary profiles. The aims of this study were to determine presence of plasmid and investigate plasmid profile of *E. coli* from clinically healthy pigs. The association between plasmid profile and resistance phenotype was also examined.

Materials and Methods

Bacterial isolates: A total of 145 *E. coli* isolates were included in this study. All of the *E. coli* strains were originated from faecal samples directly collected per rectum from clinically healthy pigs. They were isolated and kept in the strain collection of Veterinary Diagnostic Laboratory (VDL), Faculty of Veterinary Science, Chulalongkorn University in our previous study (Lay et al., 2011). All the *E. coli* strains were isolated by using the standard method (Quinn et al., 1994). Briefly, the *E. coli* strains were isolated on MacConkey (Quinn et al., 1994) and identified on Eosin Methylence Blue agar and/or classical biochemical methods (Carter and Cole, 1990). Only a single colony was collected from each positive sample and stored as 20% glycerol stocks at -80°C.

All the isolates were previously examined for antimicrobial susceptibility and class 1 integrons (Lay et al., 2011). Ninety-four percent of all isolates were resistant to at least 3 antimicrobials in different

classes and defined as multidrug resistant (MDR). Resistance rates to ampicillin, chloramphenicol, ciprofloxacin, gentamicin, streptomycin, sulfamethoxazole, tetracycline and trimethoprim were 81%, 79%, 42%, 69%, 72%, 58%, 93% and 80%, respectively. Eighty-four percent were positive to intl1 and 39% carried class 1 integrons with gene cassettes (Lay et al., 2011).

Analysis of plasmid DNA: Plasmid DNA was isolated and purified from all the *E. coli* isolates using the alkaline lysis procedure as previously described with some modifications (Liou et al., 1999). Briefly, the overnight *E. coli* culture (1.5 ml) in Luria Bertani broth (LB; Difco™, BD-Diagnostic Systems, NJ, USA) was centrifuged to obtain the bacterial cell pellets. The pellets were washed with 1 ml phosphate buffer saline (PBS, Diagxotics®, Wilton, USA) and lysed with 100 µl of 10 mg/ml lysozyme (Bio Basic Inc®, Ontario, Canada) and 200 µl cell lysis solution containing 0.2 N NaOH and 1% sodium dodecyl sulfate (SDS, Amresco®, Ohio, USA). Protein was precipitated from the cell lysates by addition of 150 µl cold potassium acetate (pH 4.8) and the residual RNA was removed by adding 2 µl of 10 mg/ml Ribonuclease A (Fermentas®, Fermentas Inc., Maryland, USA). Plasmid DNA was further purified by using 400 µl phenol: chloroform: isoamyl alcohol (25:24:1) solution, precipitated in 1 ml cold-absolute ethanol and subsequently washed with 1 ml cold 70% ethanol. Afterward, the DNA pellets were air-dried and dissolved in sterile distilled water. Purified plasmids were separated on 0.5-1% agarose gels using the horizontal gel electrophoresis system. The experiment was repeated in three separate occasions for each *E. coli* isolate.

The molecular weights of plasmids were estimated using the standard curve produced by plotting $\log_{10}$ molecular weights of known DNA sizes versus their migration distance (mm). The relative mobilities on agarose gel were used to estimate sizes of unknown plasmids. Plasmid profiles (PPs) were arranged based on their sizes and numbers observed (Kado and Liu, 1981).

Results and Discussion

The main finding of this study was a variety and widespread of plasmids among *E. coli* from clinically healthy pigs. Up to 99.3% of the total *E. coli* isolates were found to carry plasmids with sizes ranging from 0.7 Mda to 16.2 Mda (Table 1). The numbers of plasmid were found to vary from one to nine. One hundred twenty seven isolates contained more than one plasmid, while 10 isolates carried up to nine plasmids. Fifty-six different plasmid profiles (PP-1 to PP-56) were observed, of which the most common plasmid profiles were PP-5 (10 isolates) and PP-21 (10 isolates). Such variation of sizes and numbers of plasmids was previously observed in *E. coli* (Malkawi and Youssef, 1998). However, it should be noted that some plasmids that are very large or very small may not be extracted by the plasmid extraction protocol used in this study.

The predominant plasmids in this study had molecular weight of 1.3 Mda (40%), 2.6 Mda (31%) and 3.3 Mda (31%). Two largest plasmids with sizes of 16.2 Mda and 15.8 Mda were found in 26% and 21% of the isolates, respectively. This is similar to previous reports in clinical isolates of *E. coli,* where plasmids with sizes ranging from 1.3 Mda to 2 Mda and 17.2 Mda were commonly found (Jan et al., 2009). The widespread of these particular plasmids implied their ability to horizontally transfer. However, plasmid transmission was not performed in this study.

It has been well known that the *E. coli* clinical isolates usually possess multiple plasmids with different sizes and this is due to exposure to various antibiotics in treatment process (Jan et al., 2009). The presence of multiple plasmids in a single bacterial strain has been linked to prolonged use of antibiotics (Levy et al., 1976). In this study, most *E. coli* isolates were multiresistant and carried multiple plasmids. Since the *E. coli* isolates in this collection were normal flora, such wide distribution of plasmids was more likely associated with use of antibiotic-growth promoters.

Fifty-six *E. coli* isolates (39%) in PP-11, 14, 20, 21, 27, 32, 40, 41 and 50-56 carried class 1 integrons with gene cassettes. Some isolates carrying class 1 integrons with resistant cassette inserts possessed common plasmids, of which two large plasmids with the size of 16.2 Mda and 15.8 Mda were frequently found. However, the size of plasmids was diverse, indicating different combinations of genes on these plasmids. Further studies are warranted to analyze plasmid structure.

All except six isolates were multidrug resistant. However, there was no significant correlation between plasmid profiles and antimicrobial resistance patterns observed among the isolates in this collection, in accordance to a previous report in the human clinical isolates (Jan et al., 2009). This is also supported by our observation that some strains in the same plasmid profile (i.e. PP-1, 9 and 20) exhibited different resistance phenotypes. The data support the notion that the acquisition of resistance has an impact on a change of the distribution of plasmids (Platt et al., 1984). Since *E. coli* were intrinsically resistant to erythromycin, it is not surprising that all the isolates in this study exhibited resistance to this antibiotic (Andremont, 1981). Therefore, the acquisition of plasmids was unlikely to be a major contributor to widespread of erythromycin resistance.

Only one *E. coli* isolate in the present study did not harbor plasmid and was resistant up to 5 antimicrobials (i.e. AMP-CHP-ERY-TET-TRI), suggesting existence of non-plasmid borne resistance determinants. In this particular strain, antimicrobial resistance might be chromosomally encoded or medicated by other mobile genetic elements. However, such very low occurrence confirmed that plasmids played a crucial role in mediating of antimicrobial resistance among the *E. coli* isolates.

Table 1 Plasmid profiles and antimicrobial resistance patterns of the *E. coli* isolates (n=145)

PP[a]	Antimicrobial resistance pattern	No. of isolates	Class 1 integrons (No.)		Plasmid profile (Mda)
			Empty	Gene cassette[c]	
1	ERY	5	-	-	5.3, 2.6, 1.3
	CIP-ERY	1	-	-	5.3, 2.6, 1.3
	CIP-ERY-GEN	2	-	-	5.3, 2.6, 1.3
	AMP-CIP-ERY-STR	1	-	-	5.3, 2.6, 1.3
2	AMP-CHP-ERY-TET	8	8	-	2.0, 1.3
3	AMP-ERY-STR-TET	1	-	-	7.9, 5.3
4	CHP-ERY-TET-TRI	1	1	-	12.5
	AMP-ERY-GEN-SUL-TET	1	-	-	12.5
5	CHP-ERY-TET-TRI	10	10	-	15.8, 4.0, 3.3, 1.7, 1.3
6	AMP-CHP-CIP-ERY-TET	2	2	-	1.2
7	AMP-CHP-ERY-TET-TRI	5	5	-	5.3, 4.0, 3.3, 2.6, 2.0, 1.0, 0.7
8	AMP-ERY-STR-SUL-TET	5	-	-	16.2, 12.5, 2.6, 1.3
9	CHP-CIP-ERY-TET-TRI	1	1	-	15.8
	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	15.8
10	CHP-ERY-STR-TET-TRI	3	3	-	12.5, 9.9, 5.3, 2.6
11	CHP-ERY-STR-TET-TRI	3	-	3	11.2
12	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET	3	-	-	15.8, 7.9, 4.0
13	AMP-CHP-CIP-ERY-GEN-STR-TET-TRI	1	1	-	4.0
14	AMP-CHP-CIP-ERY-GEN-STR-TET-TRI	4	-	4	16.2, 11.2, 5.7
15	AMP-CHP-CIP-ERY-GEN-STR-TET-TRI	1	1	-	15.8, 1.0, 0.7
16	AMP-CHP-CIP-ERY-GEN-STR-TET-TRI	1	-	-	15.8, 1.7
17	AMP-CHP-CIP-ERY-GEN-STR-TET-TRI	1	-	-	16.2, 5.7, 3.3
18	AMP-CHP-CIP-ERY-GEN-SUL-TET-TRI	2	2	-	6.6, 3.3, 2.3, 2.0, 1.3
19	AMP-CHP-CIP-ERY-STR-SUL-TET-TRI	1	1	-	4.0, 2.6
20	AMP-CHP-CIP-ERY-STR-SUL-TET-TRI	1	1	-	16.2
	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	3	-	3	16.2
	CHP-ERY-GEN-STR-TET-TRI	1	-	1	16.2
21	AMP-CHP-CIP-ERY-STR-SUL-TET-TRI	10	-	10	16.2, 15.8, 4.0, 3.3, 2.3, 1.7, 1.3, 1.0, 0.7
22	AMP-CHP-CIP-ERY-STR-SUL-TET-TRI	1	1	-	9.9, 5.3, 4.6, 2.3
23	AMP-CHP-CIP-ERY-STR-SUL-TET-TRI	1	1	-	5.3, 2.3
24	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	16.2, 7.9, 2.3
25	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	4	3	-	11.2, 5.3, 2.6, 1.7
26	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	16.2, 4.0, 2.6, 2.0, 0.7
27	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	10	-	10	9.9, 6.6, 4.0, 2.3
28	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	4.0, 2.3, 2.0, 1.0
29	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	7.9, 5.3, 3.3
30	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	2	2	-	7.9
31	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	3	3	-	15.8, 2.6, 1.7
32	AMP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	-	1	16.2, 4.0, 3.3, 1.7
33	AMP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	9.9, 3.3, 1.3, 1.0, 0.7
34	AMP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	11.2, 4.0
35	AMP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	16.2, 9.9, 4.0, 3.3
36	AMP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	3.3, 2.0
37	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	2.6, 1.7, 0.7
38	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	4.0, 2.3, 1.3, 1.0
39	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	6.6, 3.3, 1.3, 1.0
40	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	9	-	9	6.6, 2.6, 1.3, 1.0, 0.7
41	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	-	1	16.2, 3.3, 2.0, 1.7
42	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	16.2, 7.9, 4.0, 3.3
43	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	15.8, 4.0
44	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	-	-	11.2, 6.6, 3.3, 3.0, 2.0, 1.7
45	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	9.9, 4.0, 2.3
46	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	4.0, 2.3
47	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	6.6, 3.3
48	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	6.6, 5.7
49	AMP-CHP-CIP-ERY-GEN-STR-SUL-TET-TRI	1	1	-	2.6, 1.7
50	AMP-ERY-GEN-STR-TET-TRI	3	-	3	16.2, 5.3, 3.3, 1.7
51	AMP-ERY-GEN-SUL-TET-TRI	1	-	1	5.3, 3.3
52	AMP-ERY-GEN-STR-SUL-TET-TRI	4	-	4	5.3, 3.3, 2.6
53	AMP-CHP-ERY-GEN-STR-SUL-TET-TRI	1	-	1	7.9, 4.0
54	AMP-CHP-ERY-GEN-STR-TRI	1	-	1	5.3, 4.6, 3.3, 2.6, 1.7, 1.3
55	AMP-CHP-ERY-GEN-STR-TET-TRI	3	-	3	16.2, 3.3, 1.7
56	AMP-CIP-ERY-GEN-STR-TET-TRI	1	-	1	16.2, 5.3, 1.3

[a]Plasmid profiles

[b]The *E. coli* isolates carrying empty class 1 integrons (Lay et al., 2011)

[c]The *E. coli* isolates carrying class 1 integrons with gene cassettes (Lay et al., 2011)

In conclusion, the results in this study confirm the significant role of commensal *E. coli* as reservoirs of multidrug-resistant plasmids. Antimicrobial use has a serious implication for the possible co-selection of multidrug resistance mediated by plasmids. While plasmid profile analysis has been extensively used for assessment of epidemiological information, future studies are needed to study molecular characteristics of resistant plasmids for deep understanding of their role in dissemination of antimicrobial resistance.

Acknowledgements

We thank W. Wannaprasat and K. Poonsuk, Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University for laboratory assistance. The work was supported by the 90th anniversary of Chulalongkorn University fund (Ratchadaphisek Somphot Endowment Fund). K.K.L. is a recipient of the Graduate Scholarship program for Faculty Members from Neighboring Countries, Chulalongkorn University. We also thank Novatis (Thailand) Ltd., Thailand for sample collection and bacterial strains providing.

References

Andremont, A. 1981. Reduction of the aerobic Gram negative bacterial flora of the gastro-intestinal tract and prevention of traveller's diarrhea using oral erythromycin. Ann Microbiol. 132(3): 419-427.

Carter, G.R. and Cole, J.R. 1990. Enterobacteria. In: Diagnostic Procedures in Bacteriology and Mycology. 5th ed. CA: USA. 107-128.

Dobrindt, U., Hentschel, U., Kaper, J.B. and Hacker, J. 2002. Genome plasticity in pathogenic and nonpathogenic enterobacteria. Curr Top Microbiol Immunol. 264(1): 157-175.

Fluit, A.C. and Schmitz, F.J. 2004. Resistance integrons and super-integrons. Clin Microbiol Infect. 10(4): 272-288.

Hsu, S.C., Chiu, T.H., Pang, J.C., Hsuan-Yuan, C.H., Chang, G.N. and Tsen, H.Y. 2006. Characterisation of antimicrobial resistance patterns and class 1 integrons among *Escherichia coli* and *Salmonella enterica* serovar Choleraesuis strains isolated from humans and swine in Taiwan. Int J Antimicrob Agents. 27(5): 383-391.

Jan, N., Meshram, S.U. and Kulkarni, A. 2009. Plasmid profile analysis of multidrug resistant *E. coli* isolated from UTI patients of Nagpur city, India. Rom Biotechnol Lett. 14(5): 4635-4640.

Kado, C.I. and Liu, S.T. 1981. Rapid procedure for detection and isolation of large and small plasmids. J Bacteriol. 145(3): 1365-1373.

Lay, K.K., Koowattananukul, C., Chansong, N. and Chuanchuen, R. 2011. Antimicrobial-resistance, virulence and phylogenetic characteristics of *Escherichia coli* isolates from clinically healthy swine. Foodborne Pathog Dis. Submitted.

Levy, S.B., FitzGerald, G.B. and Macone, A.B. 1976. Changes in intestinal flora of farm personnel after introduction of a tetracycline-supplemented feed on a farm. N Engl J Med. 295(11): 583-588.

Liou, J.T., Shieh, B.H., Chen, S.W. and Li, C. 1999. An improved alkaline lysis method for minipreparation of plasmid DNA. Prep Biochem Biotechnol. 29(1): 49-54.

Malkawi, H.I. and Youssef, M.T. 1998. Antibiotic susceptibility testing and plasmid profiles of *Escherichia coli* isolated from diarrhoeal patients. J Trop Pediatr. 44: 128-132.

Maynard, C., Bekal, S., Sanschagrin, F., Levesque, R.C., Brousseau, R., Masson, L., Lariviere, S. and Harel, J. 2004. Heterogeneity among virulence and antimicrobial resistance gene profiles of extraintestinal *Escherichia coli* isolates of animal and human origin. J Clin Microbiol. 42(12): 5444-5452.

Moreira, M.A., Oliveira, J.A., Teixeira, L.M. and Moraes, C.A. 2005. Detection of a chloramphenicol efflux system in *Escherichia coli* isolated from poultry carcass. Vet Microbiol. 109(1-2): 75-81.

Platt, D.J., Sommerville, J.S., Kraft, C.A. and Timbury, M.C. 1984. Antimicrobial resistance and the ecology of *Escherichia coli* plasmids. J Hyg Camb. 93: 181-188.

Quinn, P.J., Carter, M.E., Markey, B.K. and Carter, G.B. 1994. Enterobacteriaceae. In: Bacteriology. Clinical Veterinary Microbiology. S.A. Grafos (ed.) Spain: Mosby- Year Book Europe. 209-236.