Original Article

Plasmid Profiles and Antimicrobial Resistance Patterns of *Escherichia coli*

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Abstract : *Antimicrobial resistance is an important problem for treatment of infectious diseases. Transmission of resistant genes via plasmids is one mechanism of spreading of resistant bacteria.* ***Objective :*** *To investigate the plasmid profiles and antimicrobial resistance of Escherichia coli (E. coli) isolated from various sources.* ***Methods:*** *Two hundred and fifty-eight strains of E. coli isolated from various origins including chickens, dogs and cats, humans, vegetables and water samples were screened for plasmids.* ***Results :*** *Overall frequency of the isolates containing plasmids was 32.2% with the highest frequency of 55.4% for chicken isolates. The prevalence from other sources were 30.4% for edible water samples, 16.7% for dogs and cats, 9.4% for fresh vegetables and 8.7% for pet owners. The number of plasmids in each isolate ranged from one to seven and the varieties of plasmid sizes ranged from 1.0 kb to 9.4 kb. More than two plasmids were demonstrated with high occurrence (29%) in E. coli derived from chicken ceca. The small-size plasmids of 1.0 to 1.4 kb were found only in chicken. Up to 96.8% of the chicken isolates harboring plasmids was resistant to tetracycline.* ***Conclusion :*** *The high varieties of plasmid profiles with plasmid sized approximately 1.0 to 10 kb were revealed in E. coli originated from chicken ceca. However, antimicrobial resistance pattern was not related to plasmid profiles.*

Key Words: ● *Plasmid profile* ● *Escherichia coli* ● *Antimicrobial resistance*

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Introduction

An emergence of antimicrobial resistance has been recognized as a major problem of public health worldwide. An important consequence of proper use, misuse and overuse of antibiotics is an emergence and a dissemination of antimicrobial resistant bacteria in humans, animals and environments.[1] After an introduction of antimicrobials, most studies showed an increasing of the resistance in pathogenic bacteria and commensal bacteria. The commensal bacteria such as *Escherichia coli* could be a reservoir of resistance genes for pathogenic bacteria. Resistance commensal bacteria of food from animal products might be contaminated and reach humans not only by direct contact of the microorganisms but also through a consumption of food products of animal origin.[2] An occurrence of resistance in the commensal microorganisms is considered to be a good indicator for selective pressure excerted by antibiotic use in that population and for the resistance problems found in pathogens.[3] The complexity balance of micro-flora in

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different habitats within the ecosystem can potentially cause the high transferability of resistance genes among bacteria occupying the habitats. The spread of plasmid encoding an antimicrobial resistance gene in *E. coli* from chickens to human handlers or of antimicrobial-resistance microorganisms from poultry to human have been reported.[1,4]

The transmission of antibiotic resistance, often to several drugs simultaneously, from one bacterium to another is attributed to plasmids. Plasmids are self-replicating double-strand DNA circles because they contain at least one DNA sequence that serves as an origin of replication which enables the plasmid DNA to be replicated independently from chromosome. Plasmids are vary in size between <2 kb and >100 kb that can code for resistance to antimicrobial agents, disinfectants, heavy metal, cations, anions, nucleic acid-binding substances or bacteriocins, and also for metabolic or virulence properties.[5] Plasmid profiles have been reported to use as an epidemiological tool and for studying the transmission of antimicrobial resistance in several bacteria.[6,7] In an outbreak of enterohemorrhagic *E. coli* O157H7 infection, plasmid profiles were used for tracing tetracycline resistance encoding a plasmid in patient and food origins.[8]

Objective of this study was to investigate the plasmid profiles and antimicrobial resistance patterns of *E. coli* originating from chicken ceca, pets (dogs and cats), human, fresh vegetables and potable water samples.

Materials and Methods

Bacterial isolates

A total of 258 isolates of *Escherichia coli*, was isolated from chickens, pets, vegetables, waters and humans in Bangkok and its vicinity. One hundred and twelve isolates from chicken cecal contents were isolated by direct plating on MacConkey agar. Thirty-six isolates from pets (dogs and cats) and twenty-three isolates from humans (pet owners) were collected by culturing their fecal samples on the agar plates. Sixty-four isolates from fresh vegetables (lettuce, Chinese cabbage, cabbage, coriander, and bean sprout) were isolated by enriching vegetables in brilliant green bile broth before plating on MacConkey agar plates. Twenty-three isolates of potable water samples were collected by culturing the filtrates in the broth and agar plates. Five typical pink colonies of *E. coli* were selected and identified by Gram staining and biochemical tests including triple sugar iron (TSI) agar, methyl red (MR), Vogues Proskauer (VP), citrate utilization, urease and indole tests. All *E. coli* isolates were preserved in brain heart infusion broth with 15% glycerol and kept at -80^{0}C for further studies.

Antimicrobial susceptibility testing

The disk diffusion method on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI) recommendation was used for antimicrobial susceptibility testing. Seven antimicrobial agents (Oxoid, Hampshire, England) included ampicillin (AMP; 10 μg), chloramphenicol (C; 30 μg), nalidixic acid (NA; 30 μg), ciprofloxacin (CIP; 5 μg), ceftriaxone (CRO; 30 μg), tetracycline (TE; 30 μg) and trimethoprim-sulfamethoxazole 1.25/23.75 (SXT; 25 μg). *E. coli* ATCC 25922 was used as quality control organism.

Plasmid profile analysis

E. coli isolates were subcultured in Luria-Bertani broth at 37°C and 3 mL of overnight culture was performed plasmid DNA extraction by a modified of phenol-chloroform method by centrifugation at 10,000 x g for 1 min. After washing in Tris- ethylene diamine tetraacetic acid (EDTA) buffer (10mM Tris, 1mM EDTA, pH 8.0; TE), the pellet was added to the cold TE-saturated phenol. The microtube was inversely mixed and centrifuged at 10,000 x g for 10 min. The supernatant was transferred into a new microtube with adding cold isopropanol to precipitate

bacterial DNA. The pellet was washed with 70% ethanol and dissolved in 60 μL of TE buffer. Plasmid DNAs were separated by electrophoresis in 0.8% agarose gel in 1x Tris acetate EDTA buffer at a constant voltage of 100 V for 1 h. After electrophoresis, the agarose gel was stained in an ethidium bromide for 30 min and a plasmid band was visualized under an ultraviolet light. A 10 kb-DNA ladder was used as a marker to estimate plasmid sizes.

Results

Two hundred and fifty-eight isolates of *E. coli* originated from different sources were screened for plasmids. The overall frequency of *E. coli* isolates containing plasmids was 32.2% (83 isolates). The highest frequency of 55.4% (62 isolates) was found in the isolates derived from cecal contents of chickens. The prevalence of *E. coli* harboring plasmids were 16.7% (6 isolates) for pet isolates, 8.7% (2 isolates) for human isolates, 9.4% (6 isolates) for vegetable isolates and 30.4% (7 isolates) for water isolates as shown in Table 1. In pet origin, 5 from 6 isolates contained 2 plasmids with different size and antimicrobial resistance patterns. Only one type of plasmid was found in isolates from fecal samples of humans whilst in vegetable and water isolates, the number of plasmid types ranging from 1 to 5. No specific plasmid profile was observed among various sources studied. The plasmid distributions of the isolates from chicken containing 1 plasmid, 2 plasmids and ≥ 3 plasmids were shown in Table 2, Table 3 and Table 4, respectively. Upto 7 plasmid sizes occurred in the isolates with sizes ranging from 1.0 kb to 8.0 kb. The highest frequency (29%) of the isolates containing

Table 1 Plasmid profiles and antimicrobial resistance of 27 isolates of *E. coli* from various sources.

Sources	Isolates	Plasmid sizes (kb)	Drug resistance *
Pets	D1	2.0, 3.5	AMP
(n=36)	D2	8.4	AMP, TE
	D3	2.9, 4.8	AMP, TE, SXT
	D4	1.6, 3.0	AMP, TE, SXT, C, NA
	D5	2.3, 4.4	AMP, TE, SXT, C, NA, CIP
	D6	2.5, 3.2	TE
Humans	H1	4.6	AMP, TE, SXT, C, NA
(n=23)	H2	1.8	TE
Vegetables	V1	1.8, 2.6	AMP, TE, NA, CIP
(n=64)	V2	2.8	AMP, TE, SXT
	V3	1.9, 2.1, 2.6, 3.0, 3.2	TE
	V4	4.8	Not found
	V5	3.9	Not found
	V6	2.2	Not found
Waters	W1	4.2	AMP
(n=23)	W2	9.4	AMP, TE, C
	W3	1.8, 2.0	AMP, TE, SXT, C, CRO
	W4	2.5, 2.8, 3.2	AMP, TE, SXT, C, NA, CIP
	W5	4.4	AMP, TE, SXT, NA
	W6	5.1	TE
	W7	1.8, 2.6	TE, SXT, C, NA

* AMP = ampicillin; C = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; NA = nalidixic acid; SXT = sulfamethoxazole/trimethoprim; TE = tetracycline

Table 2 Plasmid sizes and antimicrobial resistance patterns of 25 isolates of *E. coli* from chickens.

Plasmid sizes (kb)	Drug resistance *
1.2	AMP, TE, NA
2.8 I 3.2	AMP, TE, NA, SXT
1.6 I 2.8 I 6.0	AMP, TE, NA, SXT, C
1.4 I 1.8 I 2.6 I 3.3 I 4.0 I 4.8 I 5.6	AMP, TE, NA, SXT, C, CIP
1.0 I 1.4 I 1.6 I 1.8 I 3.8 I 6.0 I 7.4	AMP, TE, NA, SXT, CIP
2.6 I 3.0	AMP, TE, NA, C, CIP
4.7	TE, C, NA, CIP, SXT
2.2 I 2.6	TE, NA

* AMP = ampicillin; C = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; NA = nalidixic acid; SXT = sulfamethoxazole/trimethoprim; TE = tetracycline

Table 3 Plasmid profiles (2 plasmids) and antimicrobial resistance of 17 isolates of *E. coli* from chickens.

Plasmid sizes (kb)	Drug resistance *
4.6, 5.1	NA
1.9, 4.2	TE, NA
2.2, 3.5	TE, NA, SXT
1.0, 2.0 I 3.0, 4.0	AMP, TE, NA, SXT
1.4, 2.6	TE, C, NA, CIP, SXT
4.2, 4.4	AMP, TE, C, NA, CIP
1.1, 2.1 I 2.5, 4.7	AMP, TE, NA, CIP, SXT
1.1, 1.6 I 1.3, 1.6 I 1.4, 1.8 I 2.8, 3.1 I 1.9, 3.2 I 2.8, 3.6 I 2.2, 4.4 I 1.2, 6.2	AMP, TE, C, NA, CIP, SXT

* AMP = ampicillin; C = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; NA = nalidixic acid; SXT = sulfamethoxazole/trimethoprim; TE = tetracycline

Table 4 Plasmid profiles (≥ 3 plasmids) and antimicrobial resistance of 18 isolates of *E. coli* from chickens.

Number of plasmid(s)	Plasmid sizes (kb)	Drug resistance *
3	1.0, 1.4, 8.0 I 4.0, 4.6, 5.0	AMP, TE, NA, CIP, SXT
3	1.4, 2.1, 2.5	AMP, TE, C, NA, CIP, SXT
3	1.3, 1.4, 1.6 I 1.1, 1.6, 2.0 I 1.9, 2.1, 2.8 I 1.8, 2.9, 3.3	AMP, CRO, TE, NA, CIP, SXT
3	1.4, 4.0, 5.1	TE, NA, CIP, SXT
4	2.0, 2.1, 4.0, 4.7	TE, C, NA, CIP, SXT
4	1.3, 2.2, 3.4, 3.8	AMP, TE, C, NA, SXT
4	1.4, 1.7, 1.8, 2.8	AMP, C, NA, CIP, SXT
4	1.4, 1.7, 2.2, 2.5 I 1.4, 1.6, 2.1, 6.8	AMP, TE, C, NA, CIP, SXT
5	1.8, 3.1, 4.0, 5.0, 8.0	AMP, TE, NA, CIP
5	1.6, 2.3, 2.6, 4.1, 4.7	AMP, TE, C, NA, CIP, SXT
5	1.7, 1.8, 2.1, 2.4, 2.5	AMP, TE, NA, CIP, SXT
6	1.1, 1.6, 2.5, 2.9, 5.0, 5.4	AMP, TE, C, NA, CIP, SXT
7	1.2, 1.5, 1.7, 3.3, 4.0, 5.5, 8.0	TE, C, NA, CIP, SXT

* AMP = ampicillin; C = chloramphenicol; CIP = ciprofloxacin; CRO = ceftriaxone; NA = nalidixic acid; SXT = sulfamethoxazole/trimethoprim; TE = tetracycline

more than 2 plasmids was found from chicken origin whereas the isolates from the other origins contained lower frequency of 0% to 7%. Additionally, the plasmid sizes of 1.0 kb to 1.4 kb were found only in the isolates collected from chicken ceca with 35.5% occurrence of positive-plasmid isolates. The relationship between plasmid profiles and antimicrobial resistance patterns was not observed in the study. The isolates from chickens harboring plasmids with resistance to tetracycline was detected with 96.8%.

Discussion

In this study the frequency of plasmids in isolates from chickens was higher than those found in the other origins. One explanation is that a new plasmid is added into the bacterial cells while the isolates pass inside the chicken intestines.[8] The intestinal flora of animals is a common pool of resistance bacteria that possibly transfer of resistance to other bacteria. Our results were similar to the study of plasmid profiles in *Salmonella typhimurium* derived from cattle, surface water and humans.[9] The plasmid profiles of cattle strains contained at least 3 plasmids while the profiles in isolates of human and water origins carried only one to two plasmids. The high incidence of plasmids may reflect the prophylactic and therapeutic uses of antimicrobial agents in chicken flocks. High levels of resistance to trimethoprim–sulphamethoxazole and tetracycline have been reported in *E. coli* isolates from broilers, pigs and foods[10] and most of *E. coli* isolates from chicken and swine origins were resistant to tetracyclines, aminoglycosides, and sulphonamides.[11]

Several different sizes of plasmids and high number of plasmids were revealed in this study. The possible reason is that *E. coli* isolates were collected from various sources and also from different collection sites in Bangkok and surrounding areas with a long period of study. An information available on plasmid profiles of *E. coli* isolated from chickens and other sources is limited. Plasmid patterns in faecal *E. coli* of pigs showed the number of plasmids ranged from 1 to 11 and the sizes from 1.4 kb to 204 kb.[12] The plasmid sizes ranging from 1.0 kb to 9.4 kb in this study were similar to the sizes of plasmids (1.0 kb to 10 kb) in *Salmonella* isolated from turkey ceca.[13] In this study, the high prevalence of isolates containing plasmids and the variety of plasmid profiles in *E. coli* originated from cecal contents of chickens was demonstrated. The occurrence of plasmids in *E. coli* isolated from other sources including pets, humans, vetetables and waters was also observed with relatively low frequency.

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รูปแบบพลาสมิดและการดื้อต่อสารต้านจุลชีพของเชื้อ *เอสเชอริเชีย โคไล*

วิจิตร วงค์ล่ำซำ[1], วิภาวดี เสียงล้ำ[2], วัชรี ติยะสุทธิพันธุ์[1], และ โสภณ สิริสาลี[3]

[1]ภาควิชาจุลชีววิทยาคลินิกและเทคโนโลยีประยุกต์ คณะเทคนิคการแพทย์ มหาวิทยาลัยมหิดล [2]ภาควิชาจุลชีววิทยา คณะวิทยาศาสตร์ มหาวิทยาลัยสงขลานครินทร์ [3]ภาควิชาสรีรวิทยา กองการศึกษา วิทยาลัยแพทยศาสตร์พระมงกุฎเกล้า

ความเป็นมา : *การดื้อยาต้านจุลชีพเป็นปัญหาสำคัญในการให้การรักษาโรคติดเชื้อ ซึ่งการถ่ายทอดยีนที่ควบคุมการดื้อยาโดยอาศัยพลาสมิดเป็นกลไกหนึ่งในการแพร่กระจายของเชื้อดื้อยา* ***วัตถุประสงค์ :*** *เพื่อศึกษารูปแบบพลาสมิดและการดื้อต่อสารต้านจุลชีพของเชื้อ Escherichia coli (E. coli) ที่แยกได้จากแหล่งต่างๆ* ***วิธีการศึกษา :*** *สกัดแยกพลาสมิดใน E. coli จำนวน 258 สายพันธุ์ที่แยกได้จากลำไส้ไก่ สุนัข แมว คน ผักและน้ำ นำรูปแบบพลาสมิดที่พบมาวิเคราะห์* ***ผลการศึกษา :*** *พบสายพันธุ์ที่มีพลาสมิดอยู่ทั้งหมดร้อยละ 32.2 โดยพบความถี่สูงสุดในสายพันธุ์ที่แยกจากไก่คือร้อยละ 55.4 อัตราการพบสายพันธุ์ที่มีพลาสมิดจากตัวอย่างน้ำคือร้อยละ 30.4 จากตัวอย่างสุนัขและแมวคือร้อยละ 16.7 จากผักสดร้อยละ 9.4 และจากคนร้อยละ 8.7 ขนาดของพลาสมิดพบในช่วง 1.0 ถึง 9.4 kb สายพันธุ์ที่แยกจากไส้ไก่พบพลาสมิดหลายขนาดในอัตราสูงถึงร้อยละ 29 และดื้อต่อยาเตตราไซคลินร้อยละ 96.8* ***สรุป :*** *สายพันธุ์ที่แยกได้จากไก่มีรูปแบบของพลาสมิดที่มีความหลากหลายสูงกว่าจากแหล่งอื่น โดยที่รูปแบบของการดื้อต่อสารต้านจุลชีพไม่สัมพันธ์กับรูปแบบของพลาสมิด*

Key Words: ● *รูปแบบพลาสมิด* ● *เอสเชอริเชีย โคไล* ● *การดื้อยาต้านจุลชีพ*

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