The Thai Journal of Veterinary Medicine

Volume 45
Issue 2 *June, 2015*

Article 16

6-1-2015

Antimicrobial Resistance Patterns and flaA Genotypes of Campylobacter jejuni Isolated from Contracted Broiler Farms in Eastern Thailand

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Short communication

Antimicrobial Resistance Patterns and *flaA* Genotypes of *Campylobacter jejuni* Isolated from Contracted Broiler Farms in Eastern Thailand

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Abstract

The increasing incidence of antibiotic-resistant *Campylobacter* has become a major public health concern. Since little is known about antimicrobial resistance of *Campylobacter* isolated from contracted broiler farms, the major type of farm that produces chicken meat for domestic consumption in Thailand, the objective of the present study was to determine the antimicrobial resistance patterns and genotypes of *Campylobacter* isolated from contracted broiler farms where antibiotics were routinely used in their production. Sixty-nine *Campylobacter jejuni* isolates from cloacal swabs of chickens reared in 2 small contracted broiler farms in eastern Thailand were tested for their susceptibility to 5 antimicrobial agents by the agar dilution method. Then, eighteen isolates were further genotyped by *flaA* short variable region (*flaA* SVR) sequencing. The majority of *C. jejuni* tested were resistant to ciprofloxacin (95.65%), followed by tetracycline (84.06%) and ampicillin (34.78%). Approximately 35% of the isolates were multidrug-resistant strains. The most common resistance pattern observed was CIP-TET resistance (30 isolates), followed by CIP-TET-AMP resistance (23 isolates). The main *Campylobacter* genotype found in this study was *flaA* SVR allele number 287 (8 isolates), followed by *flaA* SVR allele number 783 (5 isolates). No concordant between *flaA* SVR allele number and antibiotic resistance pattern was noticed. The high resistance rate to certain antimicrobial agents observed in the present study suggests that routine monitoring of antimicrobial resistance of *Campylobacter* in contracted broiler farms should be conducted.

Keywords: antimicrobial resistance patterns, broiler, *Campylobacter*, *flaA* SVR

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Introduction

Campylobacter is recognized as a major cause of gastroenteritis worldwide. Among warm-blooded animals that can carry *Campylobacter*, poultry are considered one of the most important reservoirs. Consumption and handling of contaminated poultry meat are regarded as major sources of foodborne campylobacteriosis (Humphrey et al., 2007). Although most *Campylobacter* infections are self-limiting, severe complications such as septicemia and Guillain-Barré syndrome (GBS) can occur (Nachamkin et al., 1998). Most campylobacteriosis cases do not require antimicrobial therapy. However, antibiotic treatment may be necessary in severe cases. Macrolides, fluoroquinolones and aminoglycosides are commonly used for the treatment of *Campylobacter* infection (Aarestrup and Engberg, 2001). Over the last decade, the incidence of antibiotic resistance in *Campylobacter* isolated from food animals and humans has increased rapidly, particularly in countries with the widespread use of antibiotics in animal agriculture (Silva et al., 2011).

Contracted broiler farm is the most common type of poultry farm that produces chicken meat for domestic consumption in Thailand (Ipsos business consulting, 2013). Over the last decades, the domestic consumption of broiler meat in Thailand has increased significantly from 61,000 metric tons in 1975 to 1,059,000 metric tons in 2014 (Indexmundi, 2014). Since antimicrobials are commonly used for therapeutic purposes in commercial broiler farms, monitoring of antimicrobial resistance in commensal and pathogenic bacteria in broilers is needed. Unfortunately, only limited information on antimicrobial resistance of *Campylobacter* isolated from contracted broiler farms in Thailand has been reported. Therefore, the aim of the present study was to determine the antimicrobial resistance patterns and genotypes of *Campylobacter* isolated from contracted broiler farms where antibiotics were routinely used in their production.

Materials and Methods

Bacterial strains: Sixty-nine *C. jejuni* isolates used in this study were from the strain collection of the Department of Veterinary Public Health, Faculty of Veterinary Science, Chulalongkorn University. These *Campylobacter* strains were isolated from cloacal swabs of chickens reared in 2 small contracted broiler farms in eastern Thailand.

Antimicrobial susceptibility testing: The agar dilution method was performed according to the Clinical and Laboratory Standards Institute (CLSI) guideline (CLSI, 2008). Five antimicrobial agents tested in this study included ampicillin, ciprofloxacin, erythromycin, gentamicin and tetracycline. All antimicrobial agents were purchased from Sigma Aldrich (Sigma, MO). The range of antimicrobial concentrations tested was as follows: ampicillin (AMP; 0.008 to 512 µg/ml), ciprofloxacin (CIP; 0.008 to 512 µg/ml), erythromycin (ERY; 0.06 to 512 µg/ml), gentamicin (GEN; 0.06 to 128 µg/ml) and tetracycline (TET; 0.06 to 512 µg/ml).

Briefly, the *Campylobacter* isolates were re-subcultured onto blood agar and incubated under a microaerobic condition at 42°C for 24 h. Then, the *Campylobacter* colonies were suspended into 0.85% saline. Each *Campylobacter* suspension was adjusted to the turbidity equivalent to 0.5 McFarland standards and inoculated onto Mueller-Hinton agar (MHA) containing 5% defibrinated sheep blood and two-fold dilution series of antimicrobials. Approximately 10^4 CFU/ml of bacterial suspensions were inoculated onto MHA. All plates were incubated under a microaerobic condition at 42°C for 24 h. After incubation, minimal inhibitory concentration (MIC) was determined. The resistance breakpoints for ciprofloxacin, erythromycin, gentamicin and tetracycline used by the U.S. National Antimicrobial Resistance Monitoring System (NARMS, 2011) and the resistance breakpoint for ampicillin used by the CLSI established guideline (CLSI, 2008) were used as *Campylobacter* resistance breakpoints in the present study. The MIC breakpoints for each antimicrobial agent were as follows: ampicillin (32 µg/ml), ciprofloxacin (4 µg/ml), erythromycin (32 µg/ml), gentamicin (8 µg/ml) and tetracycline (16 µg/ml). *Campylobacter jejuni* ATCC 33560 was used as a quality control organism. *C. jejuni* isolate that was resistant to three or more groups of antimicrobial agents was determined as multidrug resistant.

Genotyping: Five *Campylobacter* isolates of each antimicrobial resistance pattern were randomly selected and genotyped by *flaA* short variable region (*flaA* SVR) sequencing. When the antimicrobial resistance pattern had less than 5 isolates, all isolates of that pattern were selected. The *flaA* gene amplification was performed according to a previously published protocol (Meinersmann et al., 1997). PCR amplification was performed with an initial denaturation at 94°C for 1 min followed by 35 cycles of denaturation at 92°C for 30 seconds, annealing at 55°C for 90 seconds, and extension at 72°C for 2.5 min, ending with a final extension step at 72°C for 5 min. The 425 bp amplicon fragment was examined by gel electrophoresis, then the PCR product was purified (NucleoSpin® Gel and PCR Clean-up, Clontech Laboratories Inc., Germany) and sequenced. Nucleotide sequence of the isolate was submitted to an online database to identify *flaA* allele number (http://pubmlst.org/campylobacter/flaA).

Results and Discussion

Most *C. jejuni* isolates from the contracted broiler farms tested in this study were resistant to ciprofloxacin (95.65%), followed by tetracycline (84.06%) and ampicillin (34.78%). Less than 1% of erythromycin-resistant and no gentamicin-resistant *Campylobacter* were observed in the present study (Table 1). In terms of multidrug resistance, approximately 35% (24 out of 69) of the *Campylobacter* isolates were multidrug-resistant strains. The most common resistance pattern observed in this study was CIP-TET resistance (30 isolates), followed by CIP-TET-AMP resistance (23 isolates). For *flaA* SVR sequencing, 6 *flaA* SVR allele types were found (Table 2). The most common genotype found in this study was *flaA* SVR allele number 287 (8 isolates), followed by *flaA* SVR allele number 783 (5 isolates). Notably, these 2 common genotypes were detected in almost every resistance

pattern. No concordance between *flaA* SVR allele number and antibiotic resistance pattern was noticed.

The resistance rates to ciprofloxacin and tetracycline observed in this study are higher than those previously reported in Thailand (Padungtod et al., 2006; Chokboonmongkol et al., 2013). The previous study by Padungtod et al. (2006) showed that approximately 50% of *Campylobacter* were resistant to ciprofloxacin and tetracycline, while Chokboonmongkol et al. (2013) reported that around 81% and 41% of *Campylobacter* isolated from broilers in northern Thailand were ciprofloxacin-resistant and tetracycline-resistant, respectively. Unlike the aforementioned studies, more than 90% of the *Campylobacter* isolates in our study were resistant to ciprofloxacin and more than 80% were resistant to tetracycline. For ampicillin, although no ampicillin-resistant *Campylobacter* was observed in the study of Padungtod et al. (2006), around 30% of the *Campylobacter* isolates in our study and the study of Chokboonmongkol et al. (2013) were resistant to this antimicrobial agent.

Table 1 MIC distributions of 69 *C. jejuni* isolated from 2 small contracted broiler farms in eastern Thailand

Antimicrobial agent	Distribution of MIC (μg/ml)[a]														No. (%) resistant isolates
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	
Ciprofloxacin						2	12	24	24	5		1			66 (95.65)
Erythromycin	1	2	15	16	28	4	1					1			1 (0.69)
Gentamicin	1	20	11	28	8										0 (0)
Tetracycline					1		4	8	7	13	12	15	7	1	56 (84.06)
Ampicillin					4	8	20	6	6	9	14	1			24 (34.78)

[a] The grey shading indicates resistant isolates.

Table 2 Antimicrobial resistance patterns and *flaA* SVR allele numbers of *C. jejuni* isolated from 2 small contracted broiler farms in eastern Thailand

Resistance pattern[a] (No. of isolates)	*flaA* SVR allele number in each pattern[b] (No. of isolates)
CIP-TET (30)	287 (2), 783 (2), 57 (1)
CIP-TET-AMP (23)[c]	287 (1), 783 (1), 255 (1), 253 (1), 45 (1)
CIP (12)	287 (4), 255 (1)
TET (1)	783 (1)
CIP-ERY-TET-AMP (1)[c]	783 (1)
Pan-susceptible (1)	287 (1)

[a]AMP, ampicillin; CIP, ciprofloxacin; ERY, erythromycin; TET, tetracycline

[b]A maximum of 5 isolates per resistance pattern were selected for *flaA* SVR typing. If the resistance pattern had less than 5 isolates, all isolates were selected.

[c]Multidrug-resistant *Campylobacter* strain

According to the antimicrobial usage data of these 2 small contracted broiler farms, the high prevalence of fluoroquinolone resistance in *Campylobacter* in the present study is likely due to the previous use of this class of antimicrobials on the farms. This finding is consistent with previous studies which also reported that fluoroquinolone resistance could be observed in *Campylobacter* isolated from conventional broiler farms after stopping the use of this antimicrobial agent for 1-4 years (Price et al., 2007; Kuana et al., 2008; Han et al., 2009). Although tetracyclines have never been used on these broiler farms, the high prevalence of tetracycline-resistant *Campylobacter* was noticed. This finding is similar to those of Luangtongkum et al. (2006) and Thibodeau et al. (2011), which also showed that approximately 60% and 44% of *Campylobacter* isolates from organic broilers were resistant to tetracyclines even though no antimicrobial agents including tetracycline were used in these organic broiler farms, respectively. Unlike tetracycline, amoxicillin, a beta-lactam antibiotic, was commonly used to relieve the symptoms of vaccination reaction in the contracted broiler flocks from which the samples were collected. Although the previous study by Elviss et al. (2009) reported that amoxicillin treatment did not induce ampicillin-resistant *Campylobacter* in the population, our results found that around 35% of the *Campylobacter* isolates were resistant to ampicillin. This finding suggested that a routine practice of amoxicillin usage should be concerned.

In terms of *flaA* allele types, although *Campylobacter* isolates from different resistance patterns were selected for genotyping, similar *flaA* allele numbers were observed. Similarly, the study of Wittwer et al. (2005) also revealed that there was no relation between antibiotic resistance profile and genotype.

In conclusion, this study revealed the complex nature of antimicrobial resistance in *Campylobacter* isolated from contracted broiler farms. Since the high resistance rate to certain antimicrobial agents was observed in the present study, routine monitoring of antimicrobial resistance in commensal and pathogenic bacteria in contracted broiler farms

should be conducted in order to reduce the inappropriate use or overuse of antibiotics in Thai broiler production.

Acknowledgements

This study was supported by the National Science and Technology Development Agency (NSTDA) grant P-11-00757. Ms. Petcharatt Charununtakorn is a recipient of the NUI-RC scholarship, National Science and Technology Development Agency, Thailand.

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บทคัดย่อ

รูปแบบการดื้อยา และ *flaA* genotypes ของเชื้อแคมไพโลแบคเตอร์ เจจูไน ที่แยกได้จากฟาร์มไก่กระทงประกันราคาในภาคตะวันออกของประเทศไทย

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การดื้อต่อยาปฏิชีวนะของเชื้อแคมไพโลแบคเตอร์ที่เพิ่มมากขึ้นได้กลายเป็นปัญหาที่สำคัญทางสาธารณสุข แต่ข้อมูลเกี่ยวกับการดื้อยาของเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากฟาร์มไก่กระทงประกันราคา ซึ่งเป็นรูปแบบหลักของการเลี้ยงไก่กระทงเพื่อผลิตเนื้อสำหรับบริโภคภายในประเทศยังมีค่อนข้างจำกัด ดังนั้นการศึกษาครั้งนี้จึงมีวัตถุประสงค์เพื่อศึกษารูปแบบการดื้อยาและลักษณะทางพันธุกรรมของเชื้อแคมไพโลแบคเตอร์ที่แยกได้จากฟาร์มไก่กระทงประกันราคาที่มีการใช้ยาปฏิชีวนะเป็นประจำในการเลี้ยง นำเชื้อแคมไพโลแบคเตอร์ เจจูไน จำนวน 69 ตัวอย่างซึ่งแยกได้จากสวอปทวารหนักของไก่ที่เลี้ยงในฟาร์มไก่กระทงประกันราคาขนาดเล็กจำนวน 2 ฟาร์มในภาคตะวันออกของประเทศไทยมาทดสอบความไวรับต่อยาปฏิชีวนะจำนวน 5 ตัวด้วยวิธี agar dilution จากนั้นทำการศึกษาลักษณะทางพันธุกรรมของเชื้อแคมไพโล-แบคเตอร์ที่มีรูปแบบการดื้อยาแตกต่างกันออกไปจำนวน 18 ตัวอย่างโดยวิธี *flaA* short variable region (*flaA* SVR) sequencing ผลการศึกษาแสดงให้เห็นว่าเชื้อแคมไพโลแบคเตอร์ เจจูไนส่วนใหญ่ดื้อต่อ ciprofloxacin (95.65%) รองลงมา ได้แก่ การดื้อต่อ tetracycline (84.06%) และ ampicillin (34.78%) ประมาณร้อยละ 35 ของเชื้อแคมไพโลแบคเตอร์ที่แยกได้มีการดื้อต่อยาปฏิชีวนะหลายชนิดพร้อมกัน รูปแบบของการดื้อต่อยาปฏิชีวนะที่พบมากที่สุดคือ CIP-TET (30 ตัวอย่าง) และ CIP-TET-AMP (23 ตัวอย่าง) ลักษณะทางพันธุกรรมส่วนใหญ่ของเชื้อแคมไพโลแบคเตอร์ที่พบในการศึกษาครั้งนี้ ได้แก่ *flaA* SVR allele number 287 (8 ตัวอย่าง) รองลงมาคือ *flaA* SVR allele number 783 (5 ตัวอย่าง) การศึกษาครั้งนี้ไม่พบความสัมพันธ์ระหว่างลักษณะทางพันธุกรรมและรูปแบบการดื้อยาของเชื้อแคมไพโลแบค-เตอร์ อัตราการดื้อต่อยาปฏิชีวนะบางตัวที่พบค่อนข้างสูงในการศึกษาครั้งนี้ แสดงให้เห็นว่าควรมีการเฝ้าระวังการดื้อต่อยาปฏิชีวนะของเชื้อแคมไพโลแบคเตอร์ในฟาร์มไก่กระทงประกันราคาอย่างสม่ำเสมอ

คำสำคัญ: รูปแบบการดื้อยา ไก่กระทง แคมไพโลแบคเตอร์ *flaA* SVR

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