Songklanakarin J. Sci. Technol.
33 (5), 493-498, Sep. - Oct. 2011

SJST SONGKLANAKARIN JOURNAL OF SCIENCE AND TECHNOLOGY
http://www.sjst.psu.ac.th

Original Article

Serotypes, molecular and antimicrobial characteristics of *Campylobacter jejuni* isolated from chicken meats in Northeastern Thailand during December, 2007 to June, 2008

Bongkot Noppon[1,3]*, Tetsuo Asai[2], Yasushi, Kataoka[3], and Takuo Sawada[3]

[1] *Department of Veterinary Public Health, Faculty of Veterinary Medicine, Khon Kaen University, Mueang, Khon Kaen, 40002 Thailand.*

[2] *National Veterinary Assay Laboratory (NVAL), Ministry of Agriculture, Forestry and Fisheries (MAFF), Kokunbunji, 185-8511, Tokyo, Japan.*

[3] *Laboratory of Veterinary Microbiology, Faculty of Veterinary Science, Nippon Veterinary and Life Science University, Musashino, 180-8602, Tokyo, Japan.*

Received 6 May 2011; Accepted 24 August 2011

Abstract

Campylobacter species were isolated from chicken meats in northeastern Thailand from December, 2007 to June, 2008. From 1,930 samples, *Campylobacter* spp. were obtained from 556 (28.8%). *Campylobacter* species were isolated from chicken livers at higher percentages compared to the other parts of chicken meats ($p<0.001$). Among 294 *Campylobacter* isolates, 187 (63.6%) were identified as *C. jejuni*, and 107 (36.4%) as *C. coli*. The results of serotyping by Penner's method showed that serotype L (22.7%) and serotype A (18.7%) were predominant. Antimicrobial susceptibility test revealed that 90.7, 37.3, 29.3 and 13.3% of *C. jejuni* were resistant to OFLX, DOXY, EM and CP, respectively. MIC_{50}/MIC_{90} of OFLX, DOXY, EM and CP were 16/128, 4/256, 0.5/128 and 2/64 µg/ml, respectively. Precaution needs to be emphasized when attempt to use OFLX and DOXY for veterinary and human medicine due to the high percentage of resistance among *C. jejuni* isolated from chicken meat origin. These alarming figures should be notified to the general public.

Keywords: *Campylobacter jejuni*, serotypes, antimicrobial characteristics

1. Introduction

The genus *Campylobacter* is of great importance in human medicine and food safety. In addition, species in this genus are classical veterinary pathogens. Campylobacteriosis is a foodborne disease and is epidemiologically linked to the consumption of poultry products as has been noted in various investigations. High prevalence of *Campylobacter* in poultry products have been reported in developing countries (Boonmar *et al.*, 2005). Moreover, *C. jejuni* is the most commonly reported cause of gastroenteritis in humans. The association between *Campylobacter* in poultry and human enteritis is due to the persistence of this bacterium in the rearing environment of broiler chickens, in which asymptomatically colonizes the intestine and eventually contaminates the carcass (Mead, 2002).

The antibiotic resistance among zoonotic microorganisms in different countries and the impact of foodborne illnesses on consumers may become a threat to the food industry. In addition, the increase in antimicrobial resistance of *Campylobacter* may result from a failure of treatment in

* Corresponding author.
Email address: bonnop@kku.ac.th

severe cases. Antimicrobial resistance has become a major public health problem in Thailand (Boonmar *et al.*, 2005). Several investigations have been conducted in the central and northern regions of Thailand on antimicrobial susceptibility of *Campylobacter* isolates from both human and chicken. However, only a limited antimicrobial susceptibility study on *Campylobacter* species has been reported from northeastern Thailand.

The present study aims to clarify the current epidemiological situation of contamination, isolation rates, and antimicrobial profiles of *C. jejuni* in northeastern Thailand.

2. Materials and Methods

2.1 Collection of chicken meats

Chicken meat samples of gizzard, liver, heart, upper and lower wing were collected from three stores in Khon Kaen province, northeastern Thailand during December, 2007 to June, 2008.

2.2 Isolation of *Campylobacter* species from chicken meats

Isolation of the campylobacters was done by direct inoculation on *Campylobacter* mCCDA agar. After samples were put into sterile plastic bag containing 0.1% (w/v) buffered peptone water (Oxoid). The mixture of meats and buffered peptone water were macerating for 2 min and two loopfuls of the macerating fluid transferred directly onto mCCDA agar with CCDA selective supplement (SR0155E, Oxoid) and *Campylobacter* growth supplements, SR 0232E (Oxoid). Incubation was performed at 42°C for 48 hr in microaerophilic atmosphere generated by Anaerocult®C (Merck, Germany). Isolated clones were picked up and examined biochemically for catalase and oxidase productions and s-shaped or gull-wing shaped morphology for genus identifications.

2.3 *Campylobacter* species identification

Species identification of *C. jejuni* and *C. coli* was confirmed by PCR (Ishihara *et al.*, 2004; Ishihara *et al.*, 2006, Linton *et al.*, 1997).

2.4 Serotyping of *C. jejuni*

Serotypes of *C. jejuni* were determined by Penner's method using passive haemagglutination tests. Commercially available kit for serotyping of *C. jejuni* (Denka Ltd., Tokyo, Japan) was used. *Campylobacter* antisera included antibodies specific to the antigenic components as follows – A: 1 and 44; B: 2; C: 3; D: 4, 13, 16, 43 and 50; E: 5; F: 6 and 7; G: 8; I: 10; J: 11; K: 12; L: 15; N: 18; O: 19; P: 21; R: 23, 36 and 53; S: 27; U: 31; V: 32; Y: 37; Z: 38; Z_2: 41; Z_4: 45; Z_5: 52; Z_6: 55; Z_7: 57 (Chuma *et al.*, 1997; Patton *et al.*, 1985; Penner and Hennessy, 1980). One drop of *Campylobacter* antisera was allotted into microtiter plates containing sensitized chicken red blood cells coated with antigen of *C. jejuni*. Microplates were incubated for 30 min in moist chambers before assessing the result.

2.5 Antimicrobial susceptibility testing of *C. jejuni*

Broth microdilution method was used in accordance with the guideline of CLSI (CLSI, 2008). Resistance and susceptibility of *C. jejuni* to four antimicrobials namely, ofloxacin (OFLX), doxycycline (DOXY), erythromycin (EM) and chloramphenicol (CP) were also employed as a tool for determining the epidemiological relationship among *C. jejuni* isolates from Thai chicken meats. Mueller-Hinton broth, CAMHB, supplemented with 5.0% (v/v) defibrinated sheep blood was used as a medium of choice. Freshly grown isolates were suspended into 0.85% (w/v) physiological saline, and equilibrated to 0.5% McFarland standards. Four antimicrobials were allotted into further wells of the sterile 96-well microtiter plates and serially diluted until the final tested concentration. Then cell suspension was applied into each well, mixed, and incubated at 42°C for 48 hr in a microaerophilic atmosphere generated by Anaerocult®C (Merck). Interpretation of MIC for four antimicrobials was compared to those MIC cut off values according to CLSI (2008) guidelines. MIC breakpoints for resistance to OFLX, ≥ 2, DOXY, ≥ 16 (Ge *et al.*, 2003), EM, ≥ 32 (Ishihara *et al.*, 2006) and CP, ≥ 32 (Ge *et al.*, 2003) were employed.

2.6 MIC_{50} and MIC_{90} Determinations

MIC distributions and MICs at which 50 or 90% of the isolates were inhibited (MIC_{50} and MIC_{90}, respectively) for ofloxacin (OFLX), doxycycline (DOXY), erythromycin (EM), chloramphenicol (CP), were determined. The MIC_{50} and MIC_{90} indicate that 50% and 90%, respectively inhibited the organisms below that MIC.

3. Results and Discussion

3.1 Collection of chicken meats

Campylobacter species were isolated from chicken meats in Khon Kaen province, northeastern Thailand from December, 2007 to June, 2008. Among 1,930 chicken meat samples, *Campylobacter* spp. was isolated in 28.8% of the samples.

3.2 Isolation of *Campylobacter* species from chicken meat samples

Duncan's multiple range tests showed that there was no significant difference in prevalence of positive samples between the 3 stores ($p>0.05$). However, the results revealed significant differences between chicken parts, where chicken livers were the most contaminated parts ($p<0.001$). The order

Table 1. Distributions of *C. jejuni* isolated from chicken meats in northeastern Thailand

Chicken meats	% positive *Campylobacter* (n/N)			
	Total	Store 1	Store 2	Store 3
Gizzard	18.7[a] (73/390)	19.2 (23/120)	22.9 (32/140)	13.8 (18/130)
Heart	20.5[a] (80/390)	21.8 (24/110)	16.0 (24/150)	24.6 (32/130)
Liver	60.3[b] (229/380)	60.8 (73/120)	61.5 (80/130)	58.5 (76/130)
Upper wing	25.5[a] (107/420)	33.1 (43/130)	20.0 (32/160)	24.6 (32/130)
Lower wing	19.1[a] (67/350)	26.0 (26/100)	10.8 (14/130)	22.5 (27/120)
Total	28.8 (556/1,930)	32.6[a] (189/580)	25.6[a] (182/710)	28.9[a] (185/640)

[a] = superscript with the same alphabet indicates no significant difference

of contamination was liver, upper wing, heart, lower wing and gizzard (Table 1).

3.2 *Campylobacter* species identification

Due to the fragile nature of the organism, some isolates appeared on the first isolation and were identified as *Campylobacter* species. But, later, they did not show up on the plates, therefore, only isolates that had the capability to survive were used for subsequent experiments. Among 294 *Campylobacter* isolates examined, 63.6% were *C. jejuni* and 36.4% were *C. coli*.

3.3 Serotyping of *C. jejuni*

Of the 75 *C. jejuni* subjected to antimicrobial susceptibility testing, the majority were categorized into serotype L (22.7%), serotype A (18.7%) and non-typable (NT, 16.0%). Serotypes Y (9.3%), I (8.0%) ranked fourth and fifth, respectively (Table 2). Table 3 shows the relationships between serotypes and antimicrobial profiles of *C. jejuni* isolates.

3.4 Antimicrobial susceptibility testing of *C. jejuni*

Seventy five *C. jejuni* were subjected to antimicrobial susceptibility testing using the broth microdilution method. Resistance of *C. jejuni* to OFLX, DOXY, EM and CP were 90.7, 37.3, 29.3 and 13.3%, respectively (Tables 4, 5 and 6). In addition, serotype L was found most resistance OFLX (88.2%), followed by serotype A (85.7%) and none-typable (NT) (83.3%) (Table 3). Moreover, serotype L, A, Y, I, F, B, U and NT were more resistant to OFLX (Table 3). However, serotypes A, Y, U, Z_4, Z_2, D, R and L: Z_4 were more susceptible to CP than serotypes L, B, I, F, A: F and NT (Table 3). The majority were resistant to one antimicrobial (44.0%), followed by resistance to two antimicrobials (21.3%), three antimicrobials (17.3%) and four antimicrobial (8.0%) (Table 5). Additionally, the MIC_{50}/MIC_{90} values of the 75 *C. jejuni* isolates to OFLX, DOXY, EM and CP were 16/128, 4/256, 0.5/128 and 2/64 μg/ml, respectively (Table 7).

Table 2. Serotyping of *C. jejuni* isolated in northeastern Thailand using Penner's method

Serotype	No. of isolates	(%)
L	17	22.7
A	14	18.7
B	4	5.3
D	1	1.3
F	5	6.7
I	6	8.0
R	1	1.3
U	4	5.3
Y	7	9.3
Z_2	1	1.3
Z_4	1	1.3
A, F	1	1.3
L, Z_4	1	1.3
NT	12	16.0
Total	75	100.0

NT= none-typable

Table 3. Relationship between serotypes and antimicrobial susceptibility of *C. jejuni* isolates from chicken meats in northeastern Thailand

Serotypes	No. of Isolates	No. of isolates for resistance (%)			
		OFLX	DOXY	EM	CP
L	17	15	7	4	4
A	14	12	2	2	
B	4	4	3	1	1
Y	7	7	4	4	
I	6	6	3	3	1
F	5	5	2	1	1
U	4	3	1	1	
Z_4	1	1	1		
Z_2	1	1	1	1	
D	1	1	1		
R	1	1			
A, F	1	1			1
L, Z_4	1	1			
NT	12	10	3	5	2
Total	75	68 (90.7)	28 (37.3)	22 (29.3)	10 (13.3)

NT = none-typable

Table 4. Susceptibility of 75 *C. jejuni* isolates from chicken meats in northeastern Thailand

Drug	No. of isolates with MIC (µg/ml)														Resistance (%)
	≤0.125	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	>256	
OFLX	1	2	3	1				2	36	20	1	2	5	2	68 (90.7)
DOXY	5	8	3	8	3	2	99	8	12	2	3	2	2	7	28 (37.3)
EM	3		17	18	4	5	5	1		2	1	12	6	1	22 (29.3)
CP	2	3	1	11	13	28	3	4			4	3	3		10 (13.3)
Total	11	13	24	38	20	35	17	15	48	24	9	19	16	10	NA

The grey fields denote range of dilutions tested for each substance; MICs above the range are given as the concentration closest to the range, MICs equal to or lower than the lowest concentration tested are given as the lowest tested concentration; Bold vertical lines indicate microbiological cut-off values defining resistance; The highlighted fields indicate resistance; 75 isolates were tested

Table 5. % Resistance of 75 *C. jejuni* chicken meat isolates to 4 antimicrobials

Drugs	No. of resistance isolates	%
OFLX	68	90.7
DOXY	28	37.3
EM	22	29.3
CP	10	13.3

In sum, Pearson chi-square test indicated that there was no significant difference among dates of collection, chicken parts, serotypes, and stores in resistance or susceptibilities of *C. jejuni* to four antibiotics, except that isolates from Store 3 which were significantly more susceptible to CP compared to the two other stores ($p<0.05$) in northeastern

Table 6. Resistance profiles of *C. jejuni* to OFLX, DOXY, EM and CP

Resistance profiles	No of resistance isolates	%
OFLX	33	44.0
OFLX-DOXY	10	13.3
OFLX-CP	1	1.3
OFLX-EM	5	6.7
OFLX-DOXY-EM	10	13.3
OFLX-EM-CP	1	1.3
OFLX-DOXY-CP	2	2.7
OFLX-DOXY-EM-CP	6	8.0
Susceptible to all 4 antibiotics OFLX-DOXY-EM-CP	7	9.3
Total	75	100.0

Table 7. MIC_{50} and MIC_{90} of *C. jejuni* isolates in northeastern Thailand among the 4 antimicrobials

Antimicrobials	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)
OFLX	16	128
DOXY	4	256
EM	0.5	128
CP	2	64

Thailand.

Campylobacter spp. was isolated from 556 (28.8%) of 1,930 chicken meats in northeastern Thailand in 2007-2008. Due to the fastidious and slow growing nature of the organism, some isolates of *Campylobacter* species appeared in the first plating procedure, but later they did not show up on the plate. Therefore, only 294 *Campylobacter* isolates were further identified to species level and 187 (63.6%) isolates were identified as *C. jejuni* and 107 (36.4%) as *C. coli*. Of the 75 *C. jejuni* isolates subjected to antimicrobial susceptibility testing, it was noted that resistance to OFLX, DOXY, EM and CP were 90.7, 37.3, 29.3 and 13.3%, respectively. The present study showed that 37.3% of *C. jejuni* isolated from Thai chicken meats were resistant to DOXY. A slight increased resistance to CP (13.3%) was found in Thai chicken meat isolates compared to the Thai chicken faeces isolates in 2003 (Noppon *et al.*, 2009). Further, in Thailand, Padungtod *et al.* (2006) showed no resistance of *C. jejuni* from chicken faecal samples to CP in 2000-2002 isolates compared to the present study where 13.3% of chicken meat isolates were resistant. Considering EM, it was noted the 1.8% of *C. jejuni* isolates from faecal samples were resistance (Padungtod *et al.*, 2006). However, 29.3% of *C. jejuni* isolated from chicken meats were resistant to EM in the present study. Of chicken faecal isolates from northern Thailand, 55.5% were resistant to EM in 2003 (Boonmar *et al.*, 2005) and 17.0% in Thailand as a whole between the year 2001-2004, respectively (Boonmar *et al.*, 2007). The present study showed a lower rate of resistance of *C. jejuni* derived from chicken meats in northeastern Thailand compared to that of the north. However, the present study noted a higher resistance rate among *C. jejuni* isolates from chicken origins to EM compared to the overall figures for Thailand (29.3% versus 17.0%).

Serotyping of *C. jejuni* isolates from chicken meats revealed that serotypes L and A were the most commonly found types, whereas Boonmar et al. (2005) reported that serotype A was mostly found in isolates from poultry rectal swab in northern Thailand. However, in northeastern Thailand, serotypes L and A were frequently found almost in the same number i.e. 22.7% and 18.7% in chicken meat isolates, respectively. Results also showed serotype specific resistance profiles to certain antimicrobials, such as serotype L which was more resistant to OFLX (88.2%) compared to other antimicrobials. Serotype A was found to be most resistant to OFLX (85.7%), and NT- type was found to be most resistant to OFLX (83.3%). Further, serotype F was more resistant to OFLX (6.7%) than to DOXY (5.3%). Harada *et al.* (2009) suggested that serotype is a factor contributing to the prevalence of ampicillin resistance in *C. jejuni* isolates. In the present study, similar influences of serotypes of *C. jejuni* isolates were found resistance to certain antimicrobials.

In conclusion, *Campylobacter* spp. was isolated from 28.8% chicken meat samples in 2007-2008 in northeastern Thailand. Isolations from chicken livers were significantly

higher ($p<0.001$) than other parts of chicken meat samples. The highest detection in chicken liver may be attributable to the protective effect of liver tissues on the survival of the bacterium compared to other tissue types. Antimicrobial susceptibility testing revealed that resistance of OFLX, DOXY, EM and CP were 90.7, 37.3, 29.3 and 13.3%, respectively. MIC_{50}/MIC_{90} of the 75 *C. jejuni* to OFLX, DOXY, EM and CP were 16/128, 4/256, 0.5/128 and 2/64 μg/ml, respectively. Serotypes L and A were the two most frequently found types and were the most resistant to OFLX. Influences of serotypes on antimicrobial susceptibility profiles were obscure and further investigation is warranted.

References

Boonmar, S., Sangsuk, L., Suthivarakom, K., Padungtod, P. and Morita, Y. 2005. Serotypes and antimicrobial resistance of *Campylobacter jejuni* isolated from humans and animals in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health. 36,130-134.

Boonmar, S., Morita, Y., Fujita, M., Sangsuk, L., Suthivarakom, K., Padungtod, P., Maruyama, S., Kabeya, H., Kato, M., Kozawa, K., Yamamoto, S. and Kimura, H. 2007. Serotypes, antimicrobial susceptibility, and *gyr* A gene mutation of *Campylobacter jejuni* isolates from humans and chickens in Thailand. Microbiology and Immunology. 51, 531-537.

Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobials disk and dilution susceptibility tests for bacteria isolated from animal, 2nd edition. Approved standard M31-A3. Wayne, PA.: Clinical and Laboratory Standards Institute.

Ge, B., White, D. G., McDermott, P. F., Girard, W., Zhao, S., Hubert, S. and Meng, J. 2003. Antimicrobial-resistant *Campylobacter* species from retail raw meats. Applied and Environmental Microbiology. 69, 3005-3007.

Harada, K., Ozawa, M., Ishihara, K., Koike, R., Asai, T. and Ishikawa, H. 2009. Prevalence of antimicrobial resistance among serotypes of *Campylobacter jejuni* isolates from cattle and poultry in Japan. Microbiology and Immunology. 53, 107- 111.

Ishihara, K., Yamamoto, T., Satake, S., Takayama, S., Kubota, S., Negishi, H., Kojima, A., Asai, T., Sawada, T., Takahashi, T. and Tamura, Y. 2006. Comparison of *Campylobacter i*solated from humans and food-producing animals in Japan. Journal of Applied Microbiology. 100, 153-160.

Linton, D., Lawson, A. J., Owen, R. J. and Stanley, J. 1997. PCR detection, identification to species level, and fingerprinting of *Campylobacter jejuni* and *Campylobacter coli* direct from diarrheic samples. Journal of Clinical Microbiology. 35, 2568-2572.

Mead, G. C. 2002. Factors affecting intestinal colonization of poultry by *Campylobacter* and role of microflora in control. World's Poultry Science Journal. 58, 169-178.

Noppon, B., Asai, T., Kataoka, Y. and Sawada, T. 2009. Isolation and Antimicrobial Resistance of *Campylobacter* spp. from Chicken Faecal Samples in Khon Kaen and Nearby Province of Thailand. Chiang Mai Veterinary Journal. 7, 115-123.

Padungtod, P., Kaneene, J. B., Hanson, R., Morita, Y. and Boonmar, S. 2006. Anitimicrobial resistance in *Campylobacter* isolated from food animals and humans in Northern Thailand. FEMS Immunology and Medical Microbiology. 47, 217-225.