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Antimicrobial Resistance of *Campylobacter jejuni* Isolated from Chicken in Nakhon Pathom Province, Thailand

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ABSTRACT

Campylobacter jejuni isolated from retail market-chicken in Nakhon Pathom province were determined for resistance to quinolone and other antimicrobial agents by broth microdilution method. Sixty-eight *C. jejuni* strains were resistant to quinolone drugs including nalidixic acid (69.12%), norfloxacin (69.12%), ciprofloxacin (58.82%) and marbofloxacin (25.00%). High proportions of the isolates were resistant to tetracycline (77.94%), sulfamethoxazole (72.06%), kanamycin (51.47%), ampicillin 47.06% and streptomycin (42.65%). Low proportions of the isolates were found resistance to gentamycin (16.18%) and erythromycin (13.23%). Nearly 97% of the isolates were multiple resistances to more than 4 antimicrobial agents tested.

Key words: *Campylobacter jejuni*, broth microdilution method, MICs

INTRODUCTION

Infection with *Campylobacter* species has emerged worldwide as one of the leading causes of diarrhea (Rautelin *et al*., 2003). *C. jejuni* is one of the main species involved in human infection (Saenz *et al*., 2000). The genotyping and serotyping analysis revealed that poultry can be a source of *Campylobacter* infection and the contamination occurred by direct ingestion of undercooked food or cross contamination of raw poultry to other foods (Engberg *et al*.,2001).

Fluoroquinolones and macrolides have been widely used for treatment of *Campylobacter* infections (Aquino *et al*., 2002). The increasing proportions of *Campylobacter* isolates have been reported to be resistant to these drugs (Endtz *et al*., 1991). The over uses of antimicrobial agents in veterinary medicine or as feed additives might result in the emergence and spread of resistance among *Campylobacter* strains. This caused potentially serious effects on food safety and affected to both veterinary and human health (Piddock *et al*., 2000).

In this study we determined the antimicrobial resistance patterns of sixty-eight *C. jejuni* isolated from chicken in Nakhon Pathom province and evaluated the range of their minimum

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inhibitory concentrations (MICs) using broth microdilution method.

MATERIALS AND METHODS

Bacterial strains

Sixty-eight *C. jejuni* were isolated from retail market-chicken in Nakhon Pathom province. They were identified at Kamphaengsean Animal Diagnostic Laboratory, Faculty of Veterinary Medicine, Kasetsart University. These isolates were confirmed to be *C. jejuni* by using PCR assay following the method of Stucki *et al.* (1995). The isolates were cultured on Columbia agar plate with 5% lysed horse blood and were incubated at 42°C for 48 h in microaerophilic atmosphere. *C. jejuni* colonies were transferred into 5 ml of Mueller-Hinton broth and then incubated at 37°C for 24 h in microaerophilic atmosphere to produce a suspension of 6 to 7 log CFU/ml.

Antimicrobial agents

Six groups of antimicrobial agents including aminoglycoside (gentamycin, kanamycin and streptomycin), macrolide (erythromycin), penicillin (ampicillin), quinolone (ciprofloxacin, marbofloxacin, nalidixic acid and norfloxacin), sulfanamide (sulfamethoxazole) and tetracycline, were used for this study.

Test procedure

One hundred microliters of two-fold dilution of each antimicrobial agent ranging from 0.007 to 128 µl/ml was filled in each well of 96 wells sterile microtiter plates (Luber *et al*., 2003). Each well for susceptibility testing was filled with 100 µl of 6 to 7 log CFU/ml of bacterial suspension, and mixed gently. The plates were incubated at 37°C under microaerophilic condition. The MICs was evaluated at 24 hours later.

RESULTS AND DISCUSSION

Minimum Inhibitory Concentrations (MICs) were defined as the lowest concentration that exhibits no growth of *C. jejuni* by visible reading. The MICs of antimicrobial agents for *C. jejuni* is presented in Table 1.

Each of isolates had different MICs in each antimicrobial agents and different range of MICs. The MICs range of gentamycin, kanamycin, streptomycin, erythromycin, ampicillin, ciprofloxacin, marbofloxacin, norfloxacin and sulfamethoxazole were 0.03-2, 1-128, 0.12-16, 0.5-64, 0.25-64, 0.12-16, 0.007-8, 0.06-8 and 0.5-128 µg/ml, respectively. Nalidixic acid and tetracycline had the same MICs range at 0.25-128 µg/ml. The MICs values of tetracycline, nalidixic acid, kanamycin and sulfamethoxazole were higher than other antimicrobial agents.

At present, there is no internationally accepted criterion for susceptibility testing of *Campylobacter*. Resistant breakpoint following EUCAST (2000) method was used in this study. It was classified as susceptibility or resistance according to their individual MICs distribution of each agent. When two or more sub-populations were found, isolates with lower MICs were classified as susceptibility, whereas those from sub-populations with higher MICs were classified as resistance.

The distribution of MICs of antimicrobial agents against sixty-eight isolates of *C. jejuni* is shown in Figure 1. The MICs of these antimicrobial agents was a bimodal distribution. *C. jejuni* isolates were categorized as resistance when MICs of erythromycin, kanamycin, and sulfamethoxazole exceeded 32 µg/ml; ampicillin and tetracycline exceeded 16 µg/ml; nalidixic acid and streptomycin exceeded 8 µg/ml; ciprofloxacin and marbofloxacin exceeded 4 µg/ml, whereas *C. jejuni* isolates were found as low resistance, when MICs of gentamycin and norfloxacin exceeded 1 and 2 µg/ml, respectively.

Antimicrobial concentration ranges, MIC50, MIC90, breakpoints of resistance and percentage of resistance for *C. jejuni* are presented in Table 2. MIC50 and MIC90 were MIC at which 50% and 90% of the isolates were inhibited, respectively. The data indicated that sulfamethoxazole had MIC50 and MIC90 higher than other agents at 64 and 128 µg/ml, respectively,

Table 1 MICs of 11 antimicrobial agents against sixty-eight isolates of *C. jejuni* by broth microdilution method.

	MICs (µg/ml)														
Agent[1]	128	64	32	16	8	4	2	1	0.5	0.25	0.12	0.06	0.03	0.015	0.007
GEN							2	9			45	9	3		
KAN	1	9	25				27	6							
STR				8	21					35	4				
ERY		3	6			5	14	33	7						
AMP		4	8	20					35	1					
CIP				8	11	21				23	5				
MAR					3	14						7	33	10	1
NAL	1	9	7	22	8	4	9	4	2	2					
NOR					1	9	37				13	8			
SMX	12	35	2					3	16						
TET	8	7	32	6				1	10	4					

[1] GEN = gentamycin, KAN = kanamycin, STR = streptomycin, ERY = erythromycin, AMP = ampicillin, CIP = ciprofloxacin, MAR = marbofloxacin, NAL = nalidixic acid, NOR = norfloxacin, SMX = sulfamethoxazole, TET = tetracycline

[2] MICs = Minimum Inhibitory Concentrations

Table 2 Antimicrobial agents, range of MICs, MIC50, MIC90, breakpoint of resistance and percentage of resistance from sixty-eight *C. jejuni* isolates as determination by broth microdilution.

Agent	MIC (µg/ml)			Break point of resistance (µg/ml)	Percentage of resistance
	Range	MIC50	MIC90		
Gentamycin	0.03-2	0.12	1	1	16.18%
Kanamycin	1-128	32	64	32	51.47%
Streptomycin	0.12-16	0.25	16	8	42.65%
Erythromycin	0.5-64	1	32	32	13.23%
Ampicillin	0.25-64	0.5	32	16	47.06%
Ciprofloxacin	0.12-16	4	8	4	58.82%
Marbofloxacin	0.007-8	0.03	4	4	25.00%
Nalidixic acid	0.25-128	16	64	8	69.12%
Norfloxacin	0.06-8	2	8	2	69.12%
Sulfamethoxazole	0.5-128	64	128	32	72.06%
Tetracycline	0.25-128	32	128	16	77.94%

while gentamycin had lowest values at 0.12 and 1 µg/ml, respectively.

Resistance to antimicrobial agents was commonly detected among sixty eight *C. jejuni* isolates. Resistance to four quinolones including ciprofloxacin, marbofloxacin, nalidixic acid and norfloxacin was found at 58.82, 25.00, 69.12 and 69.12%, respectively. The high prevalence of quinolone resistant *C. jejuni* isolates was similar to the finding of Engberg *et al.* (2001). However, the prevalence of marbofloxacin resistant isolates of *C. jejuni* in this study was relatively low (25.00%). The reason was possibly due to marbofloxacin is not commonly used as the other drugs in this group. Endtz *et al.* (1991) reported that certain isolates of *C. jejuni* were cross-resistant to various

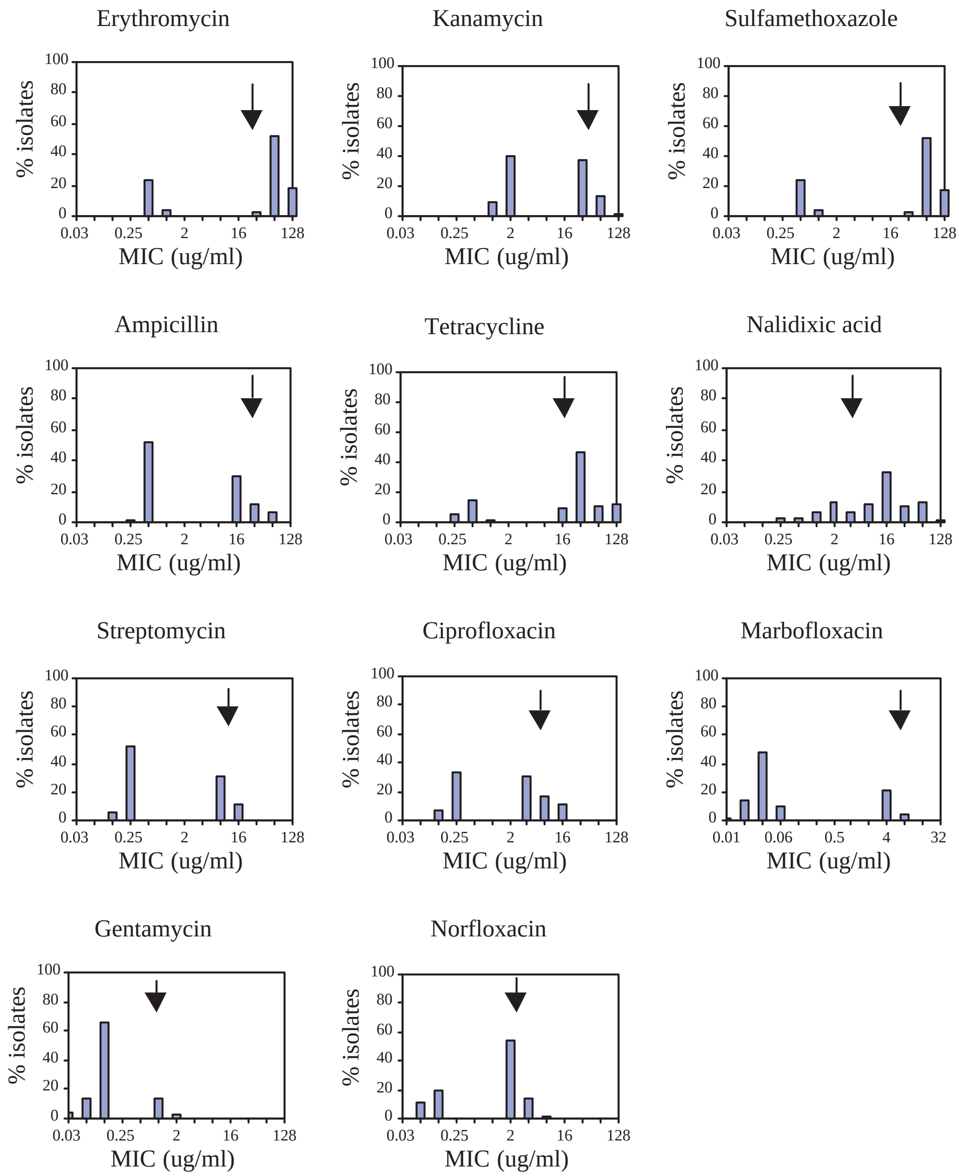

Figure 1 Distribution of MICs of 11 antimicrobial agents against sixty-eight isolates of *C. jejuni* from chicken. Arrow indicated break point of resistance.

quinolones. Most nalidixic acid-resistant isolates have been reported to be cross-resistant to one, two or three drugs in this group (Reina *et al*., 1995).

In Thailand, prevalence of quinolone resistant *Campylobacter* species in broiler farms was found to increase from 0% in 1987 to 84% in 1995 (Hoge *et al*., 1998). In addition, Daniel *et al*. (2002) found that *C. jejuni* isolated from Thai population was resistant to ciprofloxacin in high proportion (77%). Quinolone-resistant *Campylobacter* isolates in human and poultry meat were also found in other countries, including Canada (Gaunt and Piddock, 1996), Spain (Prats *et al*., 2000), Senegal (Cardinale, 2003) and Taiwan (Engberg *et al*., 2001). A fluoroquinolone-resistant *C. jejuni* infection has also been associated with foreign travel to many countries (Cardinale, 2003). It was reported that most clinical isolates of *C. jejuni* from U.S. troops in Thailand were resistant to ciprofloxacin (Murphy *et al*., 1996).

Regarding the proportion of aminoglycoside resistance, the percentage of isolate resistance in kanamycin was 51.47%, gentamycin was 16.18% and streptomycin was 42.65%. There was a report previously indicated that aminoglycosides resistance was less common in *C. jejuni* (Trieber and Taylor, 2000). *Campylobacter* species isolated from humans, pigs, cattle, and broilers were very low in resistance to streptomycin (1%) (Fallon *et al*., 2003). Moreover, it was demonstrated that there was no *Campylobacter* from chicken isolates that was resistant to aminoglycosides (Cabrita *et al*., 1992). The contrary of aminoglycosides resistance of *C. jejuni* from this work to the others was possibly due to the administration of these antimicrobial agents in veterinary medicine under certain conditions in Thailand. Therefore, resistances of these agents were found to increase in certain isolates of *C. jejuni*.

In this study, the high proportions of *C. jejuni* isolates were also resistant to other antimicrobial agents including ampicillin (47.06%), sulfamethozaxole (72.06%) and tetracycline (77.94%). These results are in accordance with the previous report that resistances to ampicillin, chloramphenicol, sulfamethozaxole and tetracycline of *C. jejuni* were commonly found in Thailand (Daniel *et al*., 2002).

Low resistant level of erythromycin and gentamycin was found in 9 and 11 isolates of *C. jejuni*, respectively. However, MIC values of these agents were different (Table 2). Since gentamycin showed a very low MIC90, it seems reasonable to consider this drug as an alternative for treatment of *C. jejuni* infections under certain conditions in Nakhon Pathom province, Thailand. Therefore, it is necessary to select the antimicrobial wisely for its effectiveness.

Resistance of *C. jejuni* isolates to a number of antimicrobial agents is presented in Table 3. Antimicrobial resistance (4 to 8 antimicrobial agents) was detected in sixty-six isolates (97.06%). Resistance to four or more of the drugs tested was

Table 3 Multi-antimicrobial resistant profiles of *C. jejuni*.

Number of antimicrobial agents	Number of resistance isolates (%)
0	2 (2.94%)
4	10 (14.70%)
5	19 (27.94%)
6	26 (38.24%)
7	9 (13.24%)
8	2 (2.94%)

defined as multiple resistances. Only two isolates of *C. jejuni* (2.94%) were susceptible to all antimicrobial agents. All of multiple resistance of *C. jejuni* was consisted of quinolone resistance. One common resistance of this group was nalidixic acid.

Most *C. jejuni* strains were resistant to multi-antimicrobial agents tested. This was possibly due to some development by genetic change or physiological adaptation of the organisms to increase antimicrobial tolerances in consequence of previous exposure to antimicrobials. Therefore, discontinuing the practice of routinely adding growth promoters to animal feeds would reduce the resistant strains of *C. jejuni* in animals in Thailand. Long-term surveillance data are needed to further evaluate the impact of any intervention in antimicrobial usages. Moreover, antimicrobial susceptibility testing methods for *Campylobacter* species are needed to be harmonized and standardized for predication that bacteria will respond to treatment from an appropriate agent.

CONCLUSION

Quinolone–resistant *Campylobactor* is a concerning issue in human medicine because of failure in treatment of diarrhea cases with quinolone. Scientists are monitoring the import meat products to protect the consumers from this organism. Most, *C. jejuni* of Thai–isolates have shown high proportion of multiple resistance to many kinds of antimicrobial agent used in the test including quinolone. Erythromycin and gentamycin are alternative drugs for treatment of these cases. Good farming practice should be implemented to reduce the usage of antimicrobials.

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LITERATURE CITED

Aquino, M.H.C., A.L.L. Filgueiras, M.C.S. Ferreira and S.S. Oliveinia. 2002. Antimicrobial resistance and plasmid profiles of *C. jejuni* and *C. coli* from human and animal sources. **Lett. Appl. Microbiol.** 34: 149-153.

Cabrita, J.J., J. Rodrigues, F. Bragance, C. Morgado and A. Goncalves. 1992. Prevalence biotypes, plasmid profiles and antimicrobial resistance of *Campylobacter* isolated from wild and domestic animals from Northeast Portugal. **J. Appl. Bacteriol.** 73: 279-285.

Cardinale, E.J., A. Dromigny, F. Tall, M. Ndiaye, M. Konte and J.D. Perrier. 2003. Fluoroquinolone susceptibility of *Campylobacter* strains, Senegal. **Antimicrob. Agents Chemother**. 58: 178-190.

Daniel, W.I., C.W. Hoge, P. Chittima, L. Bodhidatta and K.W. Hickey. 2002. Competitive antibiotic resistance of diarrheal pathogens from Vietnam and Thailand, 1996-99. **Emerg. Infect. Dis.** 8: 175-201.

Endtz, H.P., G.J. Rujis, B. Klingeren, W. H. Jansen and. R.P.Mouton. 1991. Quinolone resistance in *Campylobacter* isolated from man and poultry following the introduction of fluoroquinolone in veterinary medicine. **J. Antimicrob. Chemother.** 27: 199-208.

Engberg, J., F.M. Aurestrup, D.E. Taylor and I. Nachamkin. 2001. Quinolone and macrolide resistance in *C. jejuni* and *C. coli* resistance mechanisms and trend in human isolates. **Emerg. Infect. Dis. 7**: 24-34.

EUCAST. 2000. Terminology relating to methods for the determination of susceptibility of bacteria to antimicrobial agents. **Clin. Microbiol. Infect.** 6: 503-508.

Fallon, R., N. Sullivan, M. Maher and C. Carroll. 2003. Antimicrobial resistance of *C. jejuni* and *C. coli* isolates from broiler chickens isolated at an Irish poultry processing plant. **Lett. Appl. Microbiol.** 36: 277-281.

Gaunt, P.N. and L.J.V. Piddock. 1996. Ciprofloxacin resistant *Campylobacter* spp. In humans: an epidemiological and laboratory study. **J. Antimicrob. Chemother.** 37: 747-757.

Hoge, C.W., J.M. Gambel, C. Pitarangsri and P. Echeverria. 1998. Trends in antibiotic resistance diarrhea pathogens isolated in Thailand over 15 years. **Clin. Infect. Dis.** 26: 341-345.

Luber, P., E. Bartelt, E. Genschow, J. Wagner and H. Hahn. 2003. Comparison of broth microdilution, E Test, and agar dilution methods for antibiotic susceptibility testing of *Campylobacter jejuni* and *Campylobacter coli*. **J. Clin. Microbiol.** 41: 1062-8.

Murphy, G., P. Echeverria, L. Jackon, M. Arness and C. Leboon. 1996. Ciprofloxacin and azithromycin resistant *Campylobacter* causing travelers' s diarrhea in US troops deployed to Thai in 1994. **Clin. Infect. Dis.** 22: 864-869.

Piddock, L.J.V., V. Ricci, K. Stanley and K.Jones. 2000. Activity of antibiotics used in human medicine for *C. jejuni* isolated from farm animals and their environment in Lancashire, U.K. **J. Antimicrob. Chemother.** 46: 303-306.

Prats, G., B. Mirelis, T. Lovet, C. Munoz, E. Miro and F. Navarro. 2000. Antibiotic resistance trends in enteropathogenic bacteria isolated in 1985-1987 and 1995-1998 in Barcelona. **Antimicrob. Agents Chemother.** 44: 1140-1145.

Rautelin, H., A. Vierikko, M.L. Hanninen and M. Vaara. 2003. Antimicrobial susceptibility of *Campylobacter* strains isolated from Finnish subjects infected domestically or from those infected abroad. **Antimicrob. Agents Chemother.** 47: 102-105.

Reina, J., M.J. Ros and V. Fernandez-Baca. 1995. Resistance to erythromycin in fluoroquinolone-resistant *Campylobacter jejuni* strains isolated from human feces. **J. Antimicrob. Chemother.** 35: 351-352.

Saenz, Y., M. Zarazaga, M. Lantero and F. Baquero. 2000. Antibiotic resistance in *Campylobacter* strains isolated from animals, foods, and human in Spain in 1997-1998. **Antimicrob. Agents Chemother.** 44: 267-271.

Stucki, U., J. Frey, J. Nicolet and A.P. Burnens. 1995. Identification of *Campylobacter jejuni* on the basis of a species-specific gene that encodes a membrane protein. **J. Clin. Microbiol.** 33: 855–859.

Trieber, C.A. and D.E.Taylor. 2000. Mechanisms of antibiotic resistance in *Campylobacter*. **Am. Soc. Microbiol.** 54: 441-454.