SEROTYPES AND ANTIMICROBIAL RESISTANCE OF *CAMPYLOBACTER JEJUNI* ISOLATED FROM HUMANS AND ANIMALS IN THAILAND

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Abstract. We investigated the serotypes, distributions, and antimicrobial resistance of *Campylobacter jejuni* isolates from humans and animals as a source of infection in poultry between 2002 and 2003. A total of 50 *C. jejuni* isolates from humans and 29 *C. jejuni* isolates from poultry were studied for serotype using the Penner serotyping scheme and the drug susceptibilities of the isolates which were determined for 7 antimicrobial drugs using the disk diffusion method. Serotype B (10%), serotype E (8%) and serotype R (8%) were found in humans isolates, while serotype A (27%) was most freguently isolated from poultry, followed by serotype K (21%) and serotype C (13%). Resistance in human isolates to cephalothin was high (100%). Resistance to trimethoprim/sulfamethoxazole, ciprofloxacin, and nalidixic acid were observed in 90, 82 and 78% of isolates, respectively. Most of the isolates (88%) were susceptible to erythromycin. High levels of resistance to drugs (ciprofloxacin and nalidixic acid) were observed in the isolates from poultry. These results indicate the importance of poultry as a reservoir of *C. jejuni* infection in Thailand is limited. In addition, a high proportion of the isolates were resistant to antimicrobial drugs, particularly the quinolone group.

INTRODUCTION

Campylobacteriosis is an important infectious disease caused by the bacterial genus *Campylobacter*. *C. jejuni* and *C. coli* are the two main species isolated from the stool of diarrheal infants, animals, contaminated food and water in developing countries (Taylor, 1992; Oberhelman and Taylor, 2000) High prevalences of *Campylobacter* in poultry products have been reported in developing countries. Poultry products have been found to be an important source of *Campylobacter* in humans in both Asia and Africa (Rasrinual *et al*, 1988; Simango and Rukure, 1991). Previously, reports have shown the prevalences of *Campylobacter* spp in children in Southeast Asia range from 2.9% to 15% (Rasrinual *et al*, 1988; Varavithya *et al*, 1990). Taylor *et al* (1993) reported that 9.8% of Thai children with diarrhea had *C. jejuni*. Correlation between serotyping and biotyping from human and animal isolates indicates that Campylobacteriosis is zoonotic (Adegbola *et al*, 1990). Serotyping has been recognized as an important epidemiologic marker. Several reports have described the predominant serotypes of *Campylobacter jejuni* in humans and animals (Neilsen *et al*, 1997; Koga *et al*, 2001; Petersen *et al*, 2001), however, serotyping of *Campylobacter* isolated from humans and animals in Thailand is still obscure.

Antimicrobial resistance has become a major public health problem in Thailand. Antimicrobial resistance in both human and animal *Campylobacter* isolates has become increasingly common in developing countries (Isenbarker *et al*, 2002; Shapiro *et al*, 2001). In Thailand, quinolones are widely used for bacterial infections in humans and animals. On poultry farms, they have been used to control respiratory in-

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fections. The prevalence of quinolone resistant *Campylobacter* spp was found to increase from 0% in 1987 to 84% in 1995 (Hoge *et al*, 1998). Resistance to the quinolone group in humans isolates in Thailand has been reported (Taylor *et al*, 1993). The aim of this study was to determine the significance of poultry as a reservoir for human infection by using the data obtained from serotype distributions and antimicrobial resistance.

MATERIALS AND METHODS

Human and animal samples

Between 2002 and 2003, human fecal and blood specimens were collected at hospitals in each region. All the *Campylobacter* isolates at each of the laboratory hospitals were sent to NIH for confirmation. *Campylobacter jejuni* were selected from 45 fecal specimens and 5 blood specimens to study for serotyping. Of the human isolates, 86% were children (under 1 year of age); 54% were male. The specimens from chickens were collected by rectal swab at poultry farms in North Thailand. Sixty percent of the chicken specimens were *C. jejuni*; only twenty-nine *C. jejuni* isolates from poultry were studied for serotyping.

Isolation and identification of *C. jejuni*

Five grams of each sample was added to 45 mililiters of Preston broth (Oxoid, Hampshire, England) and streaked onto CCDA (Oxoid, France) plates. Agar plates were incubated at 42ºC for 3-5 days in microaerophilic conditions (5% O_2, 10% CO_2, 85% N_2) using a gas pack jar system (Mitsubishi Chemicals, Tokyo, Japan). One typical *Campylobacter* colony was selected for further identification by biochemical testing (positive catalase and oxidase tests, ability to hydrolyse hippurate, Gram-negative, and curved shape).

Serotyping and antimicrobial susceptibility test

A total of 50 *C. jejuni* isolates from humans and 29 *C. jejuni* isolates from poultry were selected for serotyping by the Penner serotyping scheme (Denka,Tokyo) using the passive hemagglutination test (Penner and Hennessy, 1980) and analyzed for drug susceptibility by the disk diffusion method, as described by Bauer *et al* (1966) with Mueller-Hinton agar (Difco) plates. Seven types of antimicrobial disks (BBL) containing 10 µg of ampicillin, 5 µg of ciprofloxacin, 15 µg of erythromycin, 30 µg of nalidixic acid, 30 µg of tetracycline, 30 µg of cephalothin, and 12 µg of trimethoprim/sulfamethoxazole were used. The breakpoint for the antimicrobial drugs was based on the guidelines established by the National Committee on Clinical Laboratory Standards (NCCLS, 2002).

RESULTS

The serotypes of the *C. jejuni* isolates from humans and poultry specimens are shown in Table 1. Serotype B was the predominant type in humans. Type A was the predominant type in poultry. These were followed by types E and R in humans (8%) and type K in poultry (21%). Serotype G was third in humans (6%) but was not found at all in poultry, where the third most common type was C (10%). Two isolates each were found of types A and C and one isolate each was found for types K and L in humans. In poultry, two isolates each were identified for types B and E, while only one isolate was found for type S, which was not found in humans isolates. Of the 50 isolates in humans, 28 (56%) were untypable; 7 (24%) were untypable of the 28 iso-

Table 1
Serotype of *C. jejuni* isolates from humans and poultry.

Penner's serotype	No. of positive samples (%)	
	Humans	Poultry
A	2 (4)	8 (28)
B	5 (10)	2 (7)
C	2 (4)	3 (10)
D	-	-
E	4 (8)	2 (7)
G	3 (6)	-
K	1 (2)	6 (21)
L	1 (2)	-
R	4 8)	-
S	-	1 (3)
Untypable	28 (56)	7 (24)
Total	50	29

Table 2
Antimicrobial drug resistance in *C. jejuni* isolates.

Origin of isolates	Total no. of isolates	% of isolates resistant to the following antimicrobial drugs						
		AMP	CIP	ERY	TCY	NAL	CEP	SXT
Humans	50	34	82	6	60	78	100	90
Poultry	29	25.0	88.8	55.5	50.0	83.3	67	67

AMP-Ampicillin, CIP-Ciprofloxacin, ERY-Erythromycin, TCY-Tetracycline, NAY-Nalidixic acid, CEP-Cephalothin, SXT- Trimethoprim/sulfamethoxazole

Table 3
Antimicrobial drug susceptibility of *C. jejuni* isolates.

Origin of isolates	Total no. of isolates	% of isolates susceptible to the following antimicrobial drugs						
		AMP	CIP	ERY	TCY	NAL	CEP	SXT
Humans	50	56	18	88	38	22	0	10
Poultry	29	75.0	11.1	44.4	50.0	16.6	33	33

AMP-Ampicillin, CIP-Ciprofloxacin, ERY-Erythromycin, TCY-Tetracycline, NAY-Nalidixic acid, CEP-Cephalothin, SXT- Trimethoprim/sulfamethoxazole

lates in poultry.

C. jejuni human and poultry isolate resistance rates to antimicrobial drugs are shown in Table 2. The rates of resistance in the human isolates to cephalothin, trimethoprim/sulfamethoxazole, ciprofloxacin and nalidixic acid were 100, 90, 82 and 78, respectively. The rates of resistance in the poultry isolates to ciprofloxacin, nalidixic acid, cephalothin and trimethoprim/sulfamethoxazole were 88.8, 83.3, 67 and 67%, respectively.

Table 3 shows the rates of human and poultry isolate susceptibilities to antimicrobial drugs. The highest susceptibility rate in humans was for erythromycin (88%), followed by ampicillin (56%). In contrast, the highest susceptibility rate in poultry was for ampicillin (75.0%), followed by tetracycline (50.0%).

DISCUSSION

Our study showed that serotypes B and A were the most common types found in humans and poultry isolates in Thailand between 2002 and 2003. Serotype B was the most common type in human isolates in Denmark (Petersen *et al*, 2001), Canada (Woodward and Rodgers, 2002) and Japan (Morita *et al*, 2004). Serotype A was the most common type in poultry isolates in Denmark, but not in Japan (Morita *et al*, 2004). Serotype D was not found in either Thai humans or poultry, but was the most common isolate in poultry in Japan (Morita *et al*, 2004), and was the second most common isolate in poultry in Denmark (Petersen *et al*, 2001). Serotype K was found here to be the second most common type among poultry isolates and was also the second most common type in wildlife in Denmark (Petersen *et al*, 2001). Petersen *et al* (2001) reported that there were significantly more type K isolates in wildlife than in humans and in broilers. A previous report from Japan (Koga *et al*, 2001) described that serotype O was freguently isolated from Guillain-Barre syndrome (GBS) patients. This serotype was neither found in Thai humans nor in poultry isolates in our study. Bodhidatta *et al* (2002) reported that *Campylobacter* was the most common pathogen (about 28%) in Thai children, and *C. jejuni* serotypes 36, 4 and 11 were the most common, but serotypes 4(D) and 11(J) were not found. Serotype 36 was the second most common isolate in humans in our study.

The difference in serotype distribution in the human and poultry isolates was investigated in this study, despite the limited number of poultry

isolates. These data suggest that the importance of poultry as a Campylobacter source for human infection is limited. Further studies are need to investigate the serotype distribution in other sources, such as poultry, meat products and environmental sources, to clarify the epidemiology of *Campylobacter jejuni* infection.

The rate of antimicrobial resistance in human isolates is increasing in both developed and developing countries. Previously reports have shown a marked increase in resistance to quinolones in both developed and developing countries (Steinbruckner *et al*, 2001; Feierl *et al*, 2001; Hoge *et al*, 1998; Wickin *et al*, 2001). In Thailand, ciprofloxacin resistance among *Campylobacter* species increased from 0% before 1991 to 84% in 1995. Resistance to the macrolide azithromycin was found in 7% and 15% of *Campylobacter* isolates in 1994 and 1995 (Hoge *et al*, 1998). Our results show the rates of humans isolates resistant to ciprofloxacin and nalidixic acid were 82% and 78%, respectively. These are similar to the results of Hoge *et al* (1998), Isenbarger *et al* (2002) and Bodhidatta *et al* (2002). In addition, Niyomtham and Kramomthong (2003) also reported that more than 80% of *C. jejuni* from poultry isolates were resistant to quinolones. In this study, the rates of resistance to ciprofloxacin and nalidixic acid were 88.8% and 83.3%, respectively, in poultry isolates.

Resistance to macrolides has been reported to be 90% in Spain (Mirelis *et al*, 1999). The trends over time for erythromycin resistance have remained low and stable in Japan, Canada, and Finland (Engberg *et al*, 2001). Our study showed the resistance rate in human isolates to erythromycin was only 6%, with a high susceptibility rate (88%). This is similar to a report by Bodhidatta *et al* (2002). Niyomtham and Kramomthong (2003) reported that 62% of *C. jejuni* from poultry isolates were resistant to erythromycin. The resistance rate in the poultry isolates to erythromycin in our study was 55.5%.

Increasing antimicrobial resistance rates for *Campylobacter* were noted in our study. This comes from that usage of these drugs for infections other than gastroenteritis. Self-medication often occurs in food animals and in travel to developing countries. The increased use of these drugs may account for the recent increase in resistance to these antimicrobial drugs. Surveillance of resistance rates and elucidation of modes of transmission should be futher investigated.

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