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# Emerging fluoroquinolone and macrolide resistance of *Campylobacter jejuni* and *Campylobacter coli* isolates and their serotypes in Thai children from 1991 to 2000

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## SUMMARY

This study investigated fluoroquinolone, macrolide resistances and serotype distributions among *Campylobacter jejuni* and *Campylobacter coli* isolated from children in Bangkok and rural settings during 1991–2000. Phenotypic identification, serotyping, and susceptibility testing were performed by standard microbiological procedures. The predominant serotypes of *C. jejuni* were Lior 36, 2 and 4 and of *C. coli* were Lior 8, 29 and 55. Resistance to nalidixic acid increased significantly during 1991–2000 and the frequency of isolates resistant to both nalidixic acid and ciprofloxacin in Bangkok was significantly greater than in rural settings. In 1996–2000, a significant trend was observed in *C. jejuni* isolates resistant to ciprofloxacin from Bangkok but not for macrolide resistance from both settings. In summary, fluoroquinolone resistance among *C. jejuni* and *C. coli* isolates became widespread in both Bangkok and rural settings in Thailand in the 1990s while widespread resistance to macrolides was undetected.

## INTRODUCTION

*Campylobacter* spp. are one of the leading causes of bacterial gastroenteritis worldwide with *C. jejuni* and *C. coli* being the most common species infecting both adults and children. While most outbreaks of campylobacteriosis have been reported from developed countries, sporadic and infrequent outbreaks also occur in developing countries [1, 2]. Several studies have described *Campylobacter* spp. infections in Southeast Asian countries, Laos [3], Vietnam [4], Singapore [5], Indonesia [6], Bangladesh [7] and Thailand [8], but limited information is available about their distribution in different population groups, susceptibility to antimicrobials and the serotypes involved.

Fluoroquinolone drugs, such as ciprofloxacin, are commonly used for the treatment of diarrhoea [9]. Thus, an increased incidence of fluoroquinolone resistance among human *C. jejuni* and *C. coli* isolates in Thailand is of major public health concern [4, 10, 11]. High levels of ciprofloxacin resistance in *C. jejuni* have already been reported in isolates from Thai poultry [12, 13]. Erythromycin is an effective drug for the treatment of campylobacteriosis and *Campylobacter* spp. are normally susceptible to other macrolide drugs [9]. However, resistance to erythromycin amongst *C. jejuni* and *C. coli* isolates from diarrhoeal patients in Thailand has been reported [4, 10, 14] and it is feared that campylobacter will acquire resistance to both fluoroquinolones and macrolides.

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The serotypes of *C. jejuni* and *C. coli* are traditionally determined by the detection of heat-stable or heat-labile (HL) surface antigens as described by Penner's and Lior's serotyping systems, respectively [15]. Lior's scheme is based on direct agglutination of bacterial suspensions by antiserum while the scheme of Penner utilizes passive haemagglutination. These schemes were combined by Woodward & Rodgers [15] for use in reference laboratories but antisera to Penner's types have recently become available commercially.

Given the importance of *Campylobacter* spp. as a major diarrhoeal pathogen in Thailand with increased antimicrobial resistance to public health, we describe here the development of fluoroquinolone (nalidixic acid and ciprofloxacin) and macrolide (erythromycin and azithromycin) resistance over time and the distribution of serotypes amongst *C. jejuni* and *C. coli* isolated from Bangkok and rural Thai children during 1991–2000.

## METHODS

### Source of *Campylobacter* spp. isolates

A total of 968 *C. jejuni* and 200 *C. coli* isolates was obtained from surveillance studies of diarrhoea in children aged between 0 and 10 years. These studies were conducted under protocol with informed consent by the Department of Enteric Diseases, Armed Forces Research Institute of Medical Sciences (AFRIMS) in Thailand from 1991 to 2000. The study sites were based either in Bangkok or in a rural location. Isolates of *C. jejuni* and *C. coli* from Bangkok were from the Children's Hospital and Bumrasnaradul Hospital in Nontaburi Province, located in the Bangkok Metropolitan vicinity. *C. jejuni* and *C. coli* isolates from rural settings originated from Nongkai and Tabor Hospitals located in northeastern Thailand and Nakornsrithammarat Hospital in southern Thailand. Six additional rural study sites were in Rajburi Province, 150 km west of Bangkok, and one study was conducted in Tak Province in northern Thailand.

### Isolation, identification and serology

All stool specimens were processed by a modified filtration method originally described by Steele & McDermott [16]. Briefly, a 47 mm, 0·45 $\mu$m sterile cellulose acetate membrane filter (Millipore, Bedford, MA, USA) was placed centrally on the surface of *Brucella* agar (Difco, Detroit, MI, USA) with 5% sheep blood (BAP) in 90 mm Petri dishes. Five to six drops of faecal suspension, both before and after enrichment in modified Doyle's broth [17], were applied to the membrane using care to keep drops separated. The samples were allowed to filter for 30 min in ambient air before the membranes were removed. The inoculated BAPs were incubated at 37 °C for up to 72 h in microaerobic conditions (6% $O_2$, 6% $CO_2$, 3% $H_2$ and 85% $N_2$) [18] generated by GasPak$^{TM}$ (BD Diagnostic Systems, Sparks, MD, USA) with daily examination for campylobacter appearing colonies. Isolates were characterized by Gram stain, morphology, oxidase and catalase tests, and further conventional phenotypic tests including hippurate hydrolysis [19], nitrate reduction, formation of $H_2S$ in triple sugar iron medium, oxygen tolerance, and growth at 25, 37 and 42 °C. All overnight phenotypic tests were incubated in a microaerobic environment, except for oxygen tolerance which was incubated in ambient air. *C. jejuni* and *C. coli* isolates were serotyped according to Lior's scheme with antiserum sets for 33 of the common serotypes which were obtained from the National Laboratory for Enteric Pathogens, formerly located in Ottawa, Ontario, Canada [20].

### Antimicrobial susceptibility testing

*C. jejuni* and *C. coli* were tested for antimicrobial susceptibility by disk diffusion [21] with modifications described herein. Eighteen to 48 h BAP subcultures of *C. jejuni* and *C. coli* isolates were suspended in Mueller–Hinton broth (BD Diagnostic Systems) to obtain a turbidity equivalent to a 1.0 McFarland standard, and inoculated onto Mueller–Hinton II agar (BD Diagnostic Systems) supplemented with 5% sheep blood. Isolates were tested for susceptibility to the following antibiotics: nalidixic acid (NAL, 30 $\mu$g), ciprofloxacin (CIP, 5 $\mu$g), erythromycin (ERY, 15 $\mu$g), and azithromycin (AZM, 15 $\mu$g). It should be noted that all *C. jejuni* and *C. coli* isolated during 1991–1995 were tested for susceptibility to NAL, but not all were tested against CIP, ERY and AZM during these 5 years. Disks were placed on the surface of inoculated Mueller–Hinton II agar plates and incubated at 37 °C for 24 h in a microaerobic environment. As no standardized interpretive criteria exists for *Campylobacter* spp., the inhibition zone diameters were compared against NCCLS standard guidelines for aerobic Gram-negative bacilli to interpret results as susceptible, intermediate or resistant.

Table 1. *Origin of* C. jejuni *and* C. coli *isolates from Thai children during 1991–2000*

| Location settings | Study sites | Years | Total isolates ($n=1168$)* | % species identified *C. jejuni* | *C. coli* |
|---|---|---|---|---|---|
| Bangkok | | | | | |
| Children's Hospital | 9 | 1992–2000 | 684 | 84·2 | 15·8 |
| Bumrasnaradul Hospital | 1 | 1999–2000 | 89 | 84·3 | 15·7 |
| Rural settings | | | | | |
| Rajburi | 6 | 1991–2000 | 312 | 80·8 | 19·2 |
| Tak-Maesod | 1 | 1994 | 21 | 76·2 | 23·8 |
| Nongkai-Tabor | 1 | 1999–2000 | 41 | 75·6 | 24·4 |
| Nakornsrithammarat | 1 | 2000 | 21 | 85·7 | 14·3 |

* From 1109 cases and 159 controls.

### Exclusion, inclusion criteria and data analysis

The available data for microbiological characteristics and antimicrobial susceptibility were combined. Repeat samples and multiple isolates from the same child were included only if different serotypes of *C. jejuni* or *C. coli* were isolated. The statistical analyses below were performed in STATXACT version 7 (Cytel Inc., Cambridge, MA, USA). The trend of the percent of isolates of each species in each setting resistant to each antibiotic was tested by the Cochran–Armitage trend exact *P* value procedure. The common odds ratio between resistance and setting with 95% confidence intervals was computed for each species for each antibiotic and the test for homogeneity of odds ratio was performed using the homogeneous association for $2\times2\times k$ tables procedure.

## RESULTS

### Distribution of *C. jejuni* and *C. coli* and their serotypes

The relative fraction of *C. jejuni* and *C. coli* isolates in Thai children from 1991 to 2000 is shown in Table 1. Both *C. jejuni* and *C. coli* were isolated more frequently from diarrhoea cases than from asymptomatic controls (1109 diarrhoea cases, 159 controls). *C. jejun*i represented approximately 75–85% of total numbers of *Campylobacter* spp. isolates.

### Resistance trends among *C. jejuni* and *C. coli* isolates

The temporal data for fluoroquinolone and macrolide resistance for *C. jejuni* and *C. coli* from Bangkok and rural settings is shown in Figures 1 and 2 respectively. There were significant increasing trends from 1991–2000 for NAL resistance among *C. jejuni* and *C. coli* isolates in both Bangkok and rural settings. In Bangkok (Fig. 1*a*), *C. jejuni* isolates showed a marked increase in NAL resistance from 5% in 1992 to 86–100% in 1999–2000 ($P<0{\cdot}0001$). In comparison, in rural areas, NAL resistance among *C. jejuni* isolates increased from 0% to 1% in 1991–1992 to only 47% in 1999–2000 ($P<0{\cdot}0001$). NAL resistance among *C. coli* isolates in Bangkok (Fig. 1*b*) was 28% in 1992 and increased to 80–100% in 1999–2000 ($P<0{\cdot}0001$), while among *C. coli* isolates from rural areas, NAL resistance increased from 0% in 1991–1993 to 50–60% in 1999–2000 ($P<0{\cdot}0001$).

Although resistance data for CIP, ERY, and AZM were only available for 1996–2000, the percent of *C. jejuni* isolates in Bangkok resistant to CIP (Fig. 1*c*) increased significantly from 76–80% in 1996–1997 to 100% in 2000 ($P<0{\cdot}01$). The number of *C. jejuni* isolates from Bangkok and rural settings resistant to ERY and AZM remained low from 1996 to 2000. However, resistance to these agents in *C. coli* isolates from Bangkok fluctuated from 14% to 36% over the same period but these were absent from rural areas (Fig. 2).

The common odds ratio comparing the resistance by setting was computed for *C. jejuni* and *C. coli* for each antibiotic (Tables 2 and 3). In general, the odds ratio for resistance to the fluoroquinolones was significantly greater in Bangkok than in rural settings among both *C. jejuni* and *C. coli* isolates. Despite the limited data, the odds of resistance to the macrolides, ERY and AZM, were greatest among *C. coli* isolates in Bangkok.

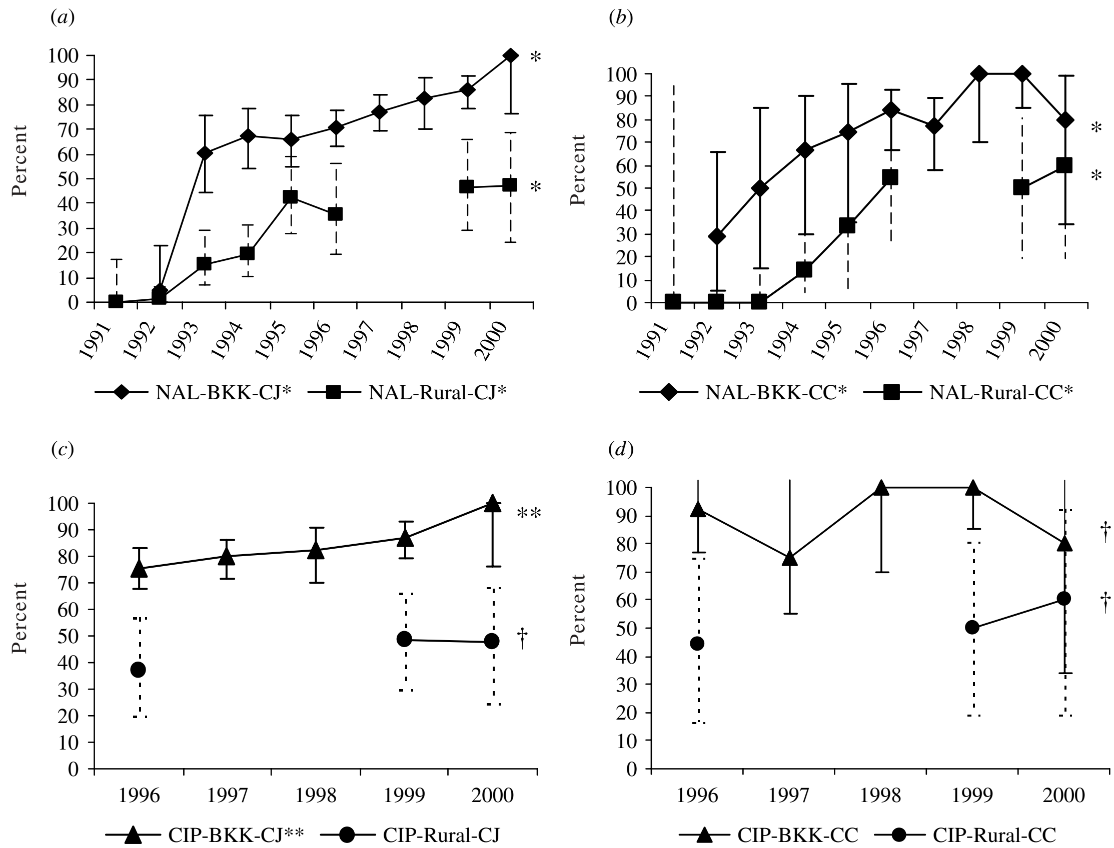


**Fig. 1.** Trends of nalidixic acid and ciprofloxacin resistance among *C. jejuni* and *C. coli* isolates from Bangkok and rural settings during 1991–2000; percent resistance by year and confidence interval (95% CI). * Cochran–Armitage trend exact *P* value (two-sided) <0·0001. ** Cochran–Armitage trend exact *P* value (two-sided) <0·01. † Cochran–Armitage trend exact *P* value (two-sided) = non-significant. NAL, Nalidixic acid; CIP, ciprofloxacin; BKK, Bangkok; CJ, *C. jejuni*; CC, *C. coli*.

### Serotype distribution

The serotype distribution of *C. jejuni* and *C. coli* isolated from Bangkok and rural settings is shown in Table 4. The most common *C. jejuni* serotype in both settings was HL 36. *C. jejuni* HL 2, 4, 7, 9 and 11 each accounted for 4–11% of isolates in both settings but *C. jejuni* HL 94 was not recovered from cases in Bangkok. For *C. coli* the most common serotypes in Bangkok were HL 8, 29, and 55 (9–16%) while HL 8, and 44 were predominant in rural areas (>10%). Twenty to 40% of all *C. jejuni* and *C. coli* isolates were not typable with the available antisera.

## DISCUSSION

This study has shown that *C. jejuni* and *C. coli* isolated from Thai children with diarrhoea exhibited significant trends of increasing resistance to NAL over 10 years. Moreover the incidence of resistance to both fluoroquinolones tested was significantly greater in isolates from Bangkok than rural settings (common OR >1). In contrast, macrolide resistance among both species in Bangkok was not significantly different from the rural setting.

Despite limited susceptibility data for CIP in isolates from 1991–1995, our results indicate a significant trend for increased resistance to CIP for *C. jejuni* isolates from Bangkok and moreover, most NAL-resistant isolates from 1996 to 2000 also showed resistance to CIP. A possible explanation for the higher fluoroquinolone resistance among city isolates is easier access to, and more frequent usage of these antimicrobial agents to treat human infections. Furthermore, these agents are widely used in veterinary medicine for treatment and prophylaxis or as growth promoters in animal and aquaculture farming system to increase production yields. Such usage may facilitate the development of antimicrobial resistance and accumulation of antimicrobials throughout the food chain. In Bangkok, meat and poultry products come from commercial farms, whereas in rural areas

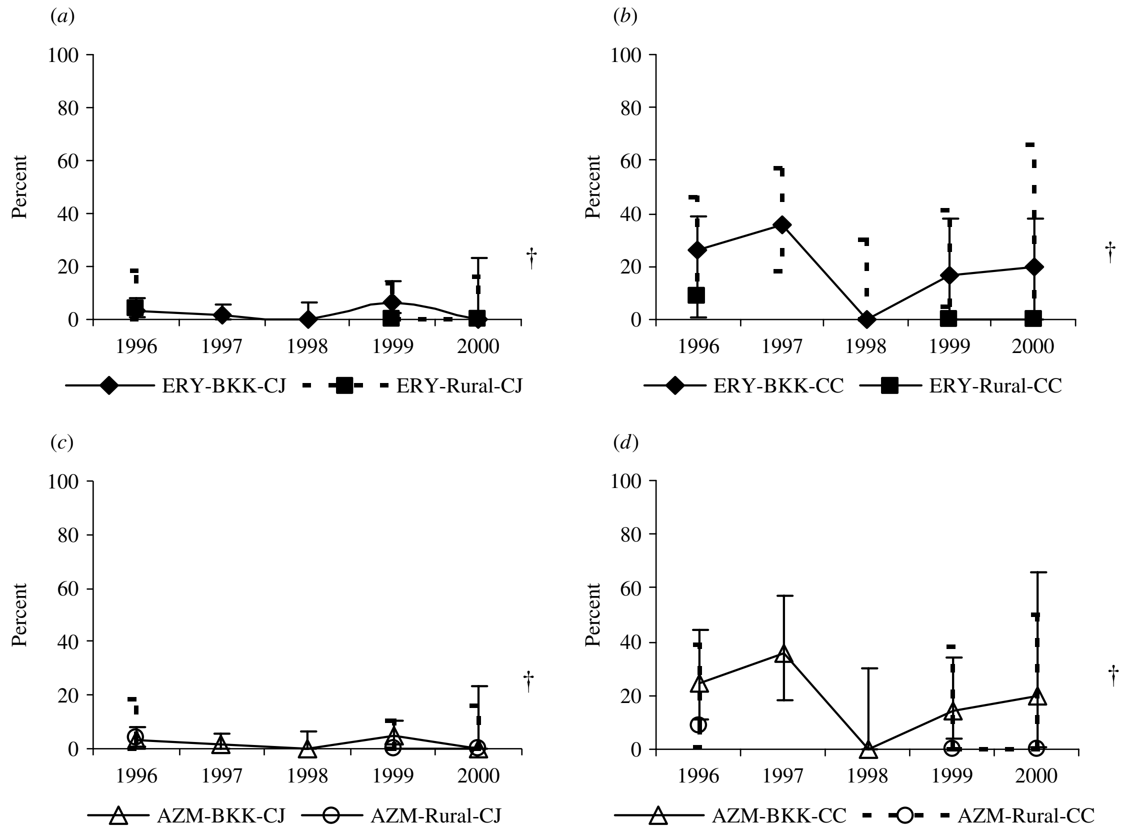


**Fig. 2.** Trends of erythromycin and azithromycin resistance among *C. jejuni* and *C. coli* isolates from Bangkok and rural settings during 1996–2000; percent resistance by year and confidence interval (95% CI). † Cochran–Armitage trend exact *P* value (two-sided) = non-significant. ERY, Erythromycin; AZM, azithromycin; BKK, Bangkok; CJ, *C. jejuni*; CC, *C. coli*.

people tend to obtain these products from their own farms where few or no antimicrobial agents are used to raise such animals. A similar increase in fluoroquinolone resistance has been observed in both human and animal *Campylobacter* spp. from Thailand as well as in a number of other countries [9, 12, 22–24].

In this study, the number of human *C. jejuni* isolates resistant to ERY was low. This contrasts with a previous report in which more than half of *C. jejuni* isolates from poultry in Thailand during 2002–2003 were resistant to ERY [25]. We were not able to collect data on food contamination associated with diarrhoea but it is unlikely that poultry was the only source of the human *C. jejuni* infections. An explanation of the more frequent macrolide resistance found in *C. coli*, compared with *C. jejuni*, might be related to both microorganisms and host. Tylosin (a macrolide derivative) is used for growth promotion in pig farms and *C. coli* are more commonly present as normal flora in pigs than in poultry [26]. This might indicate that pork was a major source of *C. coli* infection in this study. However, well-designed epidemiological follow-up studies are needed to access the degree of association between pork consumption and *C. coli* infections.

Relatively few (2·5%) of 1168 of isolates including 11 *C. jejuni* and 18 *C. coli* isolates were resistant to both fluoroquinolones and macrolides (data not shown) which is similar to our previous study of co-resistance patterns between NAL and AZM in *Campylobacter* spp. and *Salmonella* spp. isolated in Thailand [4]. No single serotype was associated with these co-resistance isolates. Subsequent antimicrobial susceptibility testing of 413 *C. jejuni* and 85 *C. coli* isolates during 2001–2005 from three studies in Thailand showed that the rate of this co-resistance among campylobacter isolates remained unchanged (L. Bodhidatta, unpublished observations). However, the finding of co-resistance to fluoroquinolones and macrolides in campylobacters is alarming as these two drug classes are the major antimicrobials currently used to treat campylobacteriosis and other enteric bacterial diarrhoea. Close monitoring of the

Table 2. *Percentage of fluoroquinolone resistance and statistical analysis between* C. jejuni *and* C. coli *isolates in Bangkok and rural settings in Thailand during 1991–2000*

| | % Nalidixic acid resistance (total tested) | | | | % Ciprofloxacin resistance (total tested) | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. jejuni* | | *C. coli* | | *C. jejuni* | | *C. coli* | |
| Year | Bangkok | Rural | Bangkok | Rural | Bangkok | Rural | Bangkok | Rural |
| 1991 | n.a.* | 0·0 (18) | n.a. | 0·0 (1) | n.a. | n.a. | n.a. | n.a. |
| 1992 | 5·0 (20) | 1·2 (81) | 28·6 (7) | 0·0 (18) | n.a. | n.a. | n.a. | n.a. |
| 1993 | 60·5 (38) | 15·0 (40) | 50·0 (6) | 0·0 (8) | n.a. | n.a. | n.a. | n.a. |
| 1994 | 67·2 (61) | 19·7 (61) | 66·7 (9) | 14·3 (21) | n.a. | n.a. | n.a. | n.a. |
| 1995 | 65·8 (79) | 42·5 (40) | 75·0 (8) | 33·3 (6) | n.a. | n.a. | n.a. | n.a. |
| 1996 | 70·8 (144) | 35·7 (28) | 83·9 (31) | 54·5 (11) | 75·7 (115) | 37·0 (27) | 92·3 (26) | 42·4 (9) |
| 1997 | 77·0 (139) | n.a. | 76·9 (26) | n.a. | 79·7 (123) | n.a. | 75·0 (24) | n.a. |
| 1998 | 82·4 (51) | n.a. | 100·0 (9) | n.a. | 82·4 (51) | n.a. | 100·0 (9) | n.a. |
| 1999 | 86·0 (107) | 46·7 (30) | 100·0 (21) | 50·0 (8) | 87·3 (102) | 48·3 (29) | 100·0 (21) | 50·0 (8) |
| 2000 | 100·0 (12) | 47·4 (19) | 80·0 (5) | 60·0 (5) | 100·0 (12) | 47·4 (19) | 80·0 (5) | 60·0 (5) |
| Test of homogeneity of association across year (exact *P* value) | | | | | | | | |
| | 0·2292 | | 0·4671 | | 0·3804 | | 0·2607 | |
| Mid *P* odds ratios (95% CI) | | | | | | | | |
| | 5·6 (3·8–8·2) | | 9·1 (3·9–22·3) | | 7·0 (3·8–13·2) | | 14·6 (3·6–76·2) | |

* Data not available.

Table 3. *Percentage of macrolide resistance and statistical analysis between* C. jejuni *and* C. coli *isolates in Bangkok and rural settings in Thailand during 1996–2000*

| | % Erythromycin resistance (total tested) | | | | % Azithromycin resistance (total tested) | | | |
|---|---|---|---|---|---|---|---|---|
| | *C. jejuni* | | *C. coli* | | *C. jejuni* | | *C. coli* | |
| Year | Bangkok | Rural | Bangkok | Rural | Bangkok | Rural | Bangkok | Rural |
| 1996 | 3·1 (98) | 4·0 (25) | 25·9 (27) | 9·1(11) | 3·1 (98) | 4·0 (25) | 25·0 (24) | 9·1 (11) |
| 1997 | 1·7 (121) | n.a.* | 36·0 (25) | n.a. | 1·6 (122) | n.a. | 36·0 (25) | n.a. |
| 1998 | 0·0 (51) | n.a. | 0·0 (9) | n.a. | 0·0 (51) | n.a. | 0·0 (9) | n.a. |
| 1999 | 6·3 (79) | 0·0 (23) | 16·7 (18) | 0·0 (7) | 4·7 (106) | 0·0 (29) | 14·3 (21) | 0·0 (7) |
| 2000 | 0·0 (12) | 0·0 (19) | 20·0 (5) | 0·0 (7) | 0·0 (12) | 0·0 (19) | 20·0 (5) | 0·0 (5) |
| Test of homogeneity of association across year (exact *P* value) | | | | | | | | |
| | 0·4071 | | 1 | | 0·4254 | | 1 | |
| Mid *P* odds ratios (95% CI) | | | | | | | | |
| | 2·2 (0·3–51·4) | | 6·1 (0·9–141·2) | | 2·2 (0·3–49·2) | | 5·4 (0·8–126·6) | |

* Data not available.

development of fluoroquinolone, macrolide resistances and their co-resistance in *Campylobacter* spp. should be undertaken in Thailand and elsewhere.

Data from the three unpublished studies cited above (L. Bodhidatta, unpublished observations) demonstrated high fluoroquinolone resistance (50–90% of isolates) but continued low macrolide resistance (0–14%) in Thailand. According to current Thai Ministry of Public Health (MOPH) guideline, quinolones are the first-line drugs of choice for the treatment of diarrhoea unless *Campylobacter* spp., is the cause of the diarrhoea, where ERY is recommended for treatment [27]. However, only a few laboratories in Thailand have the capability for isolation and identification of *Campylobacter* spp. including antimicrobial susceptibility testing. This high incidence of fluoroquinolone-resistant campylobacter isolates will inevitably result in prolongation of the illness or treatment failure in a number of cases.

Table 4. *Serotype distribution (%) of 968* C. jejuni *and 200* C. coli *isolated from Thai children in Bangkok and rural settings (1991–2000)*

| Serotype | % *C. jejuni* Bangkok (*n*=651) | % *C. jejuni* Rural (*n*=317) | Serotype | % *C. coli* Bangkok (*n*=122) | % *C. coli* Rural (*n*=78) |
|---|---|---|---|---|---|
| 36 | 27·3 (1)* | 19·9 (1) | 8 | 15·6 (1) | 14·1 (1) |
| 4 | 7·2 (2) | 6·0 (3) | 29 | 11·5 (2) | 5·1 |
| 11 | 6·8 (3) | 4·4 | 55 | 9·0 (3) | 3·8 |
| 9 | 6·0 | 6·0 (3) | 44 | 5·7 | 10·3 (2) |
| 2 | 5·4 | 10·7 (2) | 78 | 4·9 | — |
| 28 | 4·6 | 2·8 | 72 | 4·1 | 5·1 |
| 114 | 4·3 | 3·8 | 20 | 4·1 | 6·4 (3) |
| 19 | 4·1 | 1·3 | 45 | 3·3 | 2·6 |
| 7 | 3·7 | 5·7 | 46 | 2·5 | 6·4 (3) |
| 53 | 3·5 | 0·6 | 110 | 1·6 | 3·8 |
| 1 | 2·9 | 1·9 | 21 | 0·8 | 6·4 (3) |
| 17 | 2·2 | 3·2 | 47 | — | 1·3 |
| 5 | 1·2 | 0·9 | Untyped | 36·9 | 34·6 |
| 102 | 1·1 | 1·6 | — | — | — |
| 32 | 0·8 | 1·6 | — | — | — |
| 6 | 0·6 | — | — | — | — |
| 18 | 0·5 | 0·3 | — | — | — |
| 86 | 0·3 | 1·3 | — | — | — |
| 94 | — | 4·4 | — | — | — |
| 41 | — | 1·3 | — | — | — |
| 22 | — | 0·6 | — | — | — |
| 27 | — | 0·6 | — | — | — |
| 54 | — | 0·3 | — | — | — |
| 99 | — | 0·3 | — | — | — |
| 23 | — | 0·3 | — | — | — |
| 42 | — | 0·3 | — | — | — |
| 29 | 0·2 | — | — | — | — |
| Untyped | 17·4 | 19·9 | — | — | — |

* Numbers within parentheses indicate first (1), second (2) or third (3) rank in each setting.

The most common serotypes identified here, *C. jejuni* HL 36 and *C. coli* HL 8, were the five most common serotypes associated with human campylobacteriosis in a previous study of global isolates [15]. In our *C. coli* isolates, HL 29 and 44 were the second leading serotype from urban and rural settings, but they were uncommon elsewhere [15]. There was little difference in the distribution of serotypes between Bangkok and rural areas and the frequency of serologically non-typable isolates was unacceptably high. Thus serotyping alone is insufficient for studies of the epidemiology of *C. jejuni* and *C. coli* and a combination of more discriminant molecular techniques is necessary to expand our understanding of the molecular epidemiology of *Campylobacter* spp. infections in Thailand and elsewhere.

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## DECLARATION OF INTEREST

None.

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