Kasetsart J. (Nat. Sci.) 42 : 101 - 105 (2008)

Serogrouping of *Campylobacter jejuni* Isolated from Chicken Cuts, Tokyo, Japan

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ABSTRACT

Serogrouping of *Campylobacter jejuni* was conducted using Penner's method among the retail chicken cut (N = 229) isolates from 13 different outlets of which were small stores and superstores in Tokyo, Metropolis, Japan. Majority of *C. jejuni* (N = 72) in Japanese retail outlets were categorized into group B (26.39%), D (8.33%), Y (6.94%), C (4.17%), in that order. However, using this method for subspecies identification, 61.11% were typable. "Y" subspecies were the most dominant subspecies for small retail outlets and "D" was in the second ranking. Whereas "B" was the most predominated subspecies of *C. jejuni* in the superstores and "D" was second in ranking.

Key words: serogrouping, *Campylobacter jejuni*, Penner's method, chicken cuts

INTRODUCTION

Campylobacter spp. is known for its effect on human health after consumption of foods originated from animals. It is the most frequently cause of human gastroenteritis in all parts of the world as has been acknowledged worldwide. Also, traveler's diarrhea caused by *Campylobacter* has been particularly common in Thailand (Kuschner *et al*., 1995).

The genus *Campylobacter* are gram-negative, spiral, microaerophilic bacteria. Motile, with either uni- or bi-polar flagella, the organisms have a somewhat curved, rod-like appearance, and are oxidase-positive. *Campylobacter jejuni* is now recognised as one of the main causes of bacterial foodborne disease in many developed countries. At least a dozen species of *Campylobacter* have been implicated in human disease, with *C. jejuni* and *C. coli* the most common. *C. fetus* is a cause of spontaneous abortions in cattle and sheep, as well as an opportunistic pathogen in humans.

Campylobacters are carried in the intestinal tract of a wide variety of wild and domestic animals, especially birds. They can establish a temporary asymptomatic carrier state, as well as illness, in humans. This is especially prevalent in developing countries. Consumption of food and water contaminated with untreated animal or human wastes accounts for 70% of *Campylobacter*-related illnesses each year. The foods include unpasteurized milk, meats, poultry, shellfish, fruits, and vegetables (Aarestrup *et al*., 1997; Altekruse *et al*., 1999; Butzler and Oosterom, 1991; Uyttendaele *et al*., 1999).

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Campylobacter is grown on specially selective agar plates at 42°C, the normal avian body temperature. Since the colonies are oxidase positive, they will usually only grow in scanty amounts on the plates. Microaerophilic conditions are required for luxurious growth. The selective medium known as Preston's medium is used with a cocktail of antibiotics: Preston campylobacter supplement (Oxide, SR 204E) and FBP supplement (Oxoid, Campylobacter grow supplement, SR 232E), respectively.

There are 2 internationally accepted methods for serogrouping of *Campylobacter jejuni*, namely Lior and Penner's methods. The Lior schemes were used for biotyping and serogrouping campylobacter strains based on the heat-labile antigen while Penner's scheme based its protocol on heat-stable antigen.

The purposes of this study are to clarify the epidemiological diversity and molecular investigation of *C. jejuni* contamination from Japan. It is well documented that *Campylobatcter* spp. is the predominant cause of human foodborne illnesses in all parts of the world (Velazquez *et al*., 1999; Frost *et al*., 1998; Park, 2002). Discrimination of *Campylobacter* strains is an important approach to know the contamination route from the standpoint of epidemiology. Some epidemiological links may contribute to the better management or elimination program in order to avoid illnesses poses to human health as well as proper management of food producing animals for better human health. Here, Pener's scheme was used as a method of choice for serotyping of *C. jejuni* to discriminate the organism isolated from different sources.

MATERIALS AND METHODS

Isolation method: *Campylobacter* spp. was isolated from 229 chicken cut samples at retail outlets in Tokyo. Colombia Blood Agar with 5% defibrinated sheep blood was used to grow this bacterium in a microaerophilic generated by CampyPak II (BBL, UK). Plates were incubated for 48 hours for best growth. Species identifications were further conducted using PCR method. Only *Campylobacter jejuni* were subjected to serogrouping for subspecies identification of the organism. Cultures were kept at -80°C until needed. The bacteria were subcultured onto Mueller-Hinton Agar plates for best growth and used for serogrouping.

Serogrouping method: In this study, Penner serotyping using heat-stable antigens (passive haemagglutination tests, PHA) was employed for subspecies identification of *Campylobacter jejuni* after PCR identification as *C. jejuni*. The procedure followed the manufacturer's instruction. Commercial antisera, haemocytes and solutions for the extraction of antigens (Denka Seiken, Japan) were used for the tests. Several of these antisera included antibodies to multiply serotypes, as described by Penner and Hennessy (1980) and Ishihara *et al*. (2006).

RESULTS

Altogether, 7 serotypes were found in the present study i.e. B (26.39%), D (8.33%), Y (6.94%), C (4.17%), N (1.39%), I (1.39%), J (1.39%), respectively. "B" serotype was predominant among chicken cuts from Tokyo Metropolitans, especially those sold at the superstores compared to small retail outlets whereas "Y" subspecies dominated in the latter. Nevertheless, "D" serotype was found at the same numbers in both retail outlets (N = 3, each). Additionally, 5 and 6 serotypes were found among superstores and small retail outlets isolates, respectively.

Additionally, "Y" was the most dominant subspecies for small retail sale outlets and "D" was in the second ranking. Whereas "B" was the most predominated subspecies of *C. jejuni* in the superstores and "D" was second in ranking.

Table 1 Overall serogrouping of *C. jejuni* by Penner method in both small retail sale outlets and superstores.

Serogrouping	% (n/N)
Non-typable	38.89 (28/72)
B	26.39 (19/72)
D	8.33 (6/72)
Y	6.94 (5/72)
C	4.17 (3/72)
N, I, J	1.39 (1/72, 1/72, 1/72)
Total	100 (72/72)

Table 2 Comparison of percentage and numbers of serotypes of *C. jejuni* found in both small retail sale outlets and superstores.

Serogrouping	% and No. of serogroup from small retail sale outlets (n/N=18)	% and No. of serogroup from superstores (n/N=54)
Not-determine	5.55 (1/18)	12.96 (7/54)
Non-typable	33.33 (6/18)	40.74 (22/54)
B	5.55 (1/18)	33.33 (18/54)
D	16.67 (3/18)	5.55 (3/54)
Y	22.22 (4/18)	1.85 (1/54)
C	5.55 (1/18)	3.70 (2/54)
N, I, J	0, 5.55, 5.55 (0/18, 1/18, 1/18)	1.85, 0, 0 (1/54, 0/54, 0/54)
Sub-total	18/18	54/54
Total		72

DISCUSSIONS

"B" serogroup had been predominant among chicken cuts isolates especially those from superstores compared to small retail sale outlets (N = 18, 1, respectively). However, the differences of subspecies between the 2 types of retail sale outlets were not clear. It may be attributable to differences in suppliers and different sources of chicken in the area of Tokyo Metropolitan. Therefore, if an outbreak occurred, location of suppliers could be easily traced back. This may facilitate the elucidation of origin of *Campylobacter* causing human infections. All fresh chicken cuts were locally produced in Japan and distributed in Tokyo metropolis.

Ishihara *et al.* (2006) noted a similar finding of serotypes found in broilers i.e. "B", "D", "Y" serotypes were the three most frequently found serotypes in Japanese broilers. Moreover, their works were also conducted in humans and cattle isolates. Untypable numbers of *Campylobacter jejuni* were evident in this instance (33.33 and 40.74% for small retail sale outlets and superstores, respectively) as well as that noted by Ishihara *et al.* (2006) whom conducted their studies during 2001-2003. They found 12 serotypes while we found overall 7 serotypes by Penner's scheme, 6 serotypes for small retail sale outlets and 5 serotypes for superstores. Ishihara *et al.* (2006) reported the serotypes of A (no found/total no, 4/29), B (8/29), C (2/29), D (4/29), E (1/29), G (2/

29), I (0/29), J (1/29), K (1/29), L (1/29), O (0/29), R (0/29), S (0/29), U (1/29), Y (3/29), Z_2 (0/29), Z_4 (0/29), Z_5 (1/29), Z6 (0/29). In this study, we reported serotypes of B (19/72), D (6/72), Y (5/72), C (3/72), N (1/72), I (1/72), J (1/72), respectively. In our study, the overall untypable serotypes were 38.89%. Moreover, 34.48% and 38.3% were untypable in the work of Ishihara *et al*. (2006) and Nishimura *et al*. (1998), respectively.

Compared to the work done by Patton *et al*. (1985), Ishihara *et al*. (2006) and the present study, we encountered a lower typable isolates (i.e. 96.10, 75 and 61.11% in our study). Patton *et al*. (1985) stated that both Penner and Lior systems were comparable in serotyping isolates from human and nonhuman sources and for evaluating the relationship of strains collected during outbreak investigations. While 59 of 154 (38.3%) strains obtained in Japan and China were nontypable by the HS antigenic scheme, all but two of 154 (98.7%) could be typed by RFLP typing (Nishimura *et al*., 1998). It is therefore, a need for further investigation using *Fla*-A RFLP scheme in the up-coming research to clustering the isolates.

CONCLUSIONS

"B" serogroup had been predominant among chicken cuts isolates especially those from superstores compared to small retail sale outlets (N = 18, 1, respectively). However, the differences of subspecies between the 2 types of retail sale outlets were not clear. It may be attributable to differences in suppliers and different sources of chicken in the area of Tokyo Metropolitan. This may facilitate the elucidation of origin of *Campylobacter* causing human infections, especially in Japanese context.

ACKNOWLEDGEMENTS

We would like to thank National Veterinary Assay Laboratory (NVAL), Ministry of Agriculture, Forestry and Fisheries (MAFF), Tokyo, Japan and Department of Veterinary Microbiology, Nippon Veterinary and Life Science University (NVLU), Tokyo, Japan. Special thanks go to Dr. Tetsuo Asai, senior researcher at NVAL for his fruitful guidance.

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