# CHARACTERISATION OF *CAMPYLOBACTERS* FROM MALAYSIA

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**ABSTRACT**

***Eighty-five clinical and 15 poultry isolates of Campylobacter species were characterised by biotyping, serotyping and by using a radiolabelled DNA probe. A total of 80% of the isolates from both sources were identified as C. jejuni. Also amongst the clinical strains were 5 C. jejuni subsp. doylei, 7 C. coli, 3 C. lari and 8 were untypable. The similarity in the distribution of C. jejuni in the clinical and poultry isolates adds credibility to published reports of chickens being the most common source of Campylobacter infections. Although the gold standard for identification of C. jejuni is the DNA probe, serotyping is more discriminating while biotyping is the most feasible method in most laboratories.***

***Keywords:*** *Campylobacters, poultry, biotyping, serotyping, DNA probe.*

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## INTRODUCTION

*Campylobacter* species are well-established pathogens giving rise to human enteritis throughout the world. The prevalence in children, particularly in developing countries, is very high and *Campylobacters* ranked as the third most common cause of acute diarrhoeal disease after rotavirus and enterotoxigenic *Escherichia coli*[1].

These organisms exist as commensals in the intestinal tracts of a wide variety of wild and domestic animals and commercially raised poultry are known to be one of the most common sources of infection[2]. Published reports indicate the presence of *Campylobacter* infections in Malaysia and Singapore[3-5], although the reported incidence is very low. The actual incidence is probably about five to ten times higher than in developed countries but the frequency and the importance of *Campylobacter* infections in Malaysia have not been documented. In this paper we report the characterisation of both human and animal strains of *Campylobacter* species by various methods such as biotyping, serotyping, and colony hybridisation.

## MATERIALS AND METHODS

A total of 100 *Campylobacter* strains were studied, of which 85 were from diarrhoeic stools of patients from the University Hospital, Kuala Lumpur. Eight strains were from poultry before sale at a minimarket in Kuala Lumpur and seven strains from the rectal swabs of egg-laying chickens at a farm in the University of Malaya. Control strains of *C. jejuni, C. coli* and *C. lari* were obtained from the Tokyo Metropolitan Research Laboratory of Public Health.

The bacterial isolates were identified as belonging to the genus *Campylobacter* by routine bacteriological techniques, such as Gram stain, motility, growth temperature requirements, oxidase, catalase and nitrate reduction tests. In addition, antibiotic sensitivity testing was carried out by the disc diffusion method using nalidixic acid (30µg disc) and cephalothin (75µg disc). The strains were then stored at -70°C in nutrient broth supplemented with 15% glycerol.

Biochemical characterisation was carried out using the modified Lior biotyping scheme[6] which included the rapid hippurate hydrolysis test, DNA hydrolysis test, ability to grow in the presence of Trimethylamine N-oxide (TMAO) dihydrate and the production of hydrogen sulphide ($H_2S$) in triple sugar iron agar and brucella broth cultures using lead acetate strips[7].

Serotyping of some of the strains was carried out by the Public Health Laboratory, Withington Hospital, Manchester, UK using the passive haemagglutination method of Penner and Hennessy[8].

For DNA hybridisation, a specific probe for the identification of *C. jejuni*, as described by Korolik et al[9] was obtained in the form of plasmid pMO2005 in *E.coli* DH 1 as the host. Large scale extraction of the plasmid was performed[10] which was then digested with *Hind*III according to the manufacturer's (Bethesda Research Laboratories) instructions. A 1.65 kb fragment to be used as probe was extracted from a low gelling temperature agarose gel. The fragment was radiolabelled with $\alpha$-$^{32}$P dATP (Amersham) to a final specific activity of 5 x$10^7$ to 5x$10^8$ cpm/µg using nick translation.

Colony hybridisation was performed by transferring 24 hour cultures of *Campylobacters* to nylon membranes (Hybond N, Amersham) by using toothpicks as applicators. The colonies were lysed in denaturing solution (0.5M NaCl, 0.5M NaOH) followed by neutralisation using 1.5M NaCl, 0.5M Tris and 1mM EDTA. The membranes were washed in 2 x SSC solution, air dried and the chromosomal DNA fixed by exposure to ultra-violet light for 2-3 minutes. They were stored at room temperature.

Hybridisation at the highest stringency was carried out as described by Moseley et al[11]. Briefly, prehybridisation was performed for 24 hours at 65°C with 0.5% bovine serum albumin in 2 x SSC solution. Hybridisation was carried out for 24 hours at 42°C in 6 x Blotto/formamide solution using the previously prepared labelled DNA probe in a fresh volume

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of the same solution. The membranes were washed, air dried and exposed to X-ray film for 24-48 hours. Reference strains were included as controls.

## RESULTS

All 85 clinical isolates and all 15 poultry isolates had the following common characteristics : vibrio form, motile, non-fermentation or oxidation of glucose, and no $H_2S$ production in triple sugar iron agar. All cultures grew at 37°C and 42°C and not at 25°C. They were oxidase and catalase positive and resistant to cephalothin. The nitrate reductase test was positive for all strains except for five clinical strains.

The results of biochemical characterisation are shown in Table I. Of the 85 clinical strains, 67 (79%) were identified as *C. jejuni*, 7(8%) as *C. coli*, 3(4%) as *C. lari* and 8(9%) were only positive for $H_2S$ production[7] and therefore could not be classified. Of the 15 poultry isolates, 12 were identified as *C. jejuni*, 2 as *C. lari* and one as *C. coli*.

The 67 *C. jejuni* clinical isolates were further biotyped into 4 different groups: 37 belonged to biotype IV being positive for hippurate hydrolysis, $H_2S$ production and DNA hydrolysis, biotype II was the next largest group with 23 isolates, being hippurate and DNA hydrolysis positive; 4 strains belonged to biotype III (hippurate hydrolysis and $H_2S$ production positive) and only 3 belonged to biotypeI (hippurate hydrolysis positive). Amongst the 12 poultry isolates of *C. jejuni*, 7 belonged to biotypeIV. The 7 clinical isolates of *C. coli* could be differentiated as 2 strains belonging to biotypeI, (all 3 tests being negative) and 5 strains into biotypeII (only DNA hydrolysis positive). Of the 3 *C. lari* clinical isolates, one was biotypeI, ($H_2S$ positive) and 2 were biotypeII ($H_2S$ production and DNA hydrolysis positive).

None of the isolates were able to grow in the TMAO medium except for the *C. lari* reference strain.

The distribution of the serotypes of 26 clinical isolates of *C. jejuni* is shown in Table II. A total of 15 different serotypes were identified with 4 strains being untypable. The most common types observed were serotypes 2 and 3 and those belonging to serotype 4, 13, 50.

The 67 clinical isolates of *C. jejuni* plus an additional strain that was hippurate negative were able to hybridise with the specific DNA probe. All 12 poultry isolates were also confirmed to be *C. jejuni* by this method.

**Table I – Biochemical characterisation of 85 clinical isolates of *Campylobacter* spp.**

| | *C. jejuni* * | *C. coli* # | *C. lari* @ |
|---|---|---|---|
| Hippurate hydrolysis | 67 | 0 | 0 |
| $H_2S$ production | 41 | 0 | 3 |
| DNA hydrolysis | 60 | 5 | 3 |
| Nalidixic acid resistance | 0 | 0 | 3 |

* n = 67
\# n = 7
@ n = 3

**Table II – Distribution of serotypes of *C. jejuni***

| Serotype | Number |
|---|---|
| 1 | 1 |
| 2 | 3 |
| 3 | 3 |
| 4 | 1 |
| 9 | 2 |
| 23 | 1 |
| 24 | 1 |
| 4,13,50 | 3 |
| 9 (37) | 1 |
| 50 | 1 |
| 2W31 | 1 |
| 31 | 1 |
| 3,31 | 1 |
| 15,46 | 1 |
| 4,44 | 1 |
| Not typable | 4 |
| Total | 26 |

## DISCUSSION

The differentiation of thermophilic *Campylobacters* into species and biotypes provides valuable markers for epidemiological investigations.

The ratio of *C. jejuni* isolates to *C. coli* isolates from human varies from one geographical area to another. In our study *C. jejuni* (80%) was found to be the major isolate, both from humans as well as poultry. These findings confirm published reports of *C. jejuni* being the most common species giving rise to human infection[2] although in Poland and in the Central African Republic, *C. coli* is more frequently isolated[12,13].

Although only 8% and 5% of our total isolates were identified as *C. coli* and *C. lari* respectively, this may have been an underestimate due to these strains being more sensitive to the antibiotic used in the selective media for primary isolation[14].

The gastrointestinal tract of chickens and hens are generally colonised by *Campylobacter* species, and therefore they form the most common source for human infection all over the world[3]. In a preliminary study we had a 30% isolation frequency of *Campylobacters* from chickens and the similarity of the species distribution both in humans and poultry in our study suggests poultry as the most common source of *Campylobacter* infection.

There were 5 clinical strains that were negative for nitrate reductase and thus fit into the *C. jejuni* subsp. *doylei* category[15]. On the other hand, 8 clinical strains could not be speciated and perhaps require more specific tests as it has been reported that these so-called untypable strains are more common in developing countries[16].

The detection of 15 serotypes amongst the 26 clinical isolates of *C. jejuni* is an indication of good discrimination by this method for epidemiological studies although 15.5% of the isolates were not typable with the available sera. Nevertheless these findings are similar to published reports from developing countries[17]. However, serotyping is only available in reference laboratories as the 60-108 antisera required are not available commercially.

The use of a specific DNA probe to speciate the *Campylobacters* is of course the gold standard, but the methodology is expensive and requires special expertise which is not available in most developing countries. But the biochemical tests that were used in this study gave 100% concordance with the DNA probe and we recommend the routine use of these as they are simple, cost-effective and easily available, provided some of the disadvantages and discrepancies are kept in mind. More recently developed techniques such as pulsed-field gel electrophoresis and

multilocus enzyme electrophoresis[18] may offer more specific alternatives to the epidemiological studies of *Campylobacters*, but again special equipment and expertise are required.


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