

CAMPYLOBACTER JEJUNI AS A CAUSE OF DIARRHOEA IN KUALA LUMPUR, MALAYSIA

Y. S. LIM
M. JEGATHESAN
Y. H. WONG

SUMMARY

The incidence of Campylobacter jejuni in patients with and without diarrhoea was studied in Kuala Lumpur, Malaysia. C. jejuni was recovered from 3.8% and 4.3% of diarrhoeal stools of children and adults, respectively. From the patients without diarrhoea, the relative isolation rates for children and adults were 2.6% and 0%, respectively. Dual infections occurred in two children, with Salmonella and enteropathogenic Escherichia coli being the other enteric pathogen in each case. Cary-Blair medium was found to be an effective transport medium in recovering C. jejuni. Campylobacter enteritis occurred in patients of various age groups, indicating that this organism should be sought routinely by diagnostic laboratories in faecal specimens from patients with diarrhoea.

INTRODUCTION

With the development of a selective coproculture technique by Butzler *et al.*, in 1973,¹ the role of *Campylobacter jejuni* as a causal agent of infective diarrhoea has been established. Since 1977, due to the work of Skirrow,² this organism has been

increasingly recognized as a cause of human diarrhoea.

In developed countries, *C. jejuni* has been isolated from the stools of 3–14% of patients with diarrhoea.^{3,4,5} Studies on enteric campylobacteriosis in developing countries such as Africa,⁶⁻¹⁰ Bangladesh,^{11,12} Brazil,¹³ India¹⁴ and Indonesia¹⁵ have also indicated the widespread prevalence of this infection in children and adults, giving isolation rates ranging from 5–35%.

In Malaysia, however, very little information on the incidence of *C. jejuni* in patients with diarrhoea is available. This investigation was therefore undertaken to determine the prevalence of *C. jejuni* in diarrhoeal cases and to compare it with the other commonly implicated enteric pathogens.

MATERIALS AND METHODS

Study Population

Four groups were included in this study. Groups A and B consisted of children (below ten years) and adults, respectively, with diarrhoea. They comprised both in-patients and out-patients of the General Hospital Kuala Lumpur, and patients of general practitioners situated around Kuala Lumpur. Groups C and D included hospital patients (children and adults, respectively) who were admitted for illnesses other than diarrhoea.

Y.S. Lim, MSc
M. Jegathesan, MBBS, MRCPATH
Y.H. Wong, BSc (Hons)
Department of Bacteriology
Institute for Medical Research
Jalan Pahang, Kuala Lumpur

Bacteriological Methods

Stool specimens (either fresh or in Cary-Blair transport medium¹⁶) received from July 1983 to June 1984 were examined for *C. jejuni* by inoculating onto blood agar base No. 2 (CM 271; Oxoid), supplemented with 10% ox blood and antibiotics (SR 98; Oxoid). The plates were incubated at 42°C for 48 hr in an atmosphere of 5% oxygen, 10% carbon dioxide, and 85% hydrogen, achieved by using the gas-generating kit system for campylobacters (BR 56, Oxoid) in an anaerobic jar.

Faecal samples from groups A and B were also cultured by standard procedures¹⁷ for *Salmonella*, *Shigella*, *Vibrio cholerae* and *Vibrio parahaemolyticus*. The presence of enteropathogenic *Escherichia coli* was determined from stool specimens of group A only.

Suggestive *Campylobacter*-like colonies were Gram-stained (using carbol fuchsin as the counter stain) and checked for motility (by a wet mount preparation), oxidase and catalase reactions. All isolates which were Gram-negative and showed a curved or spiral morphology, oxidase and catalase positive and exhibited darting motility, were presumptively identified as *Campylobacter*. The isolates were confirmed as *C. jejuni* if they reduced nitrates to nitrites, did not utilize glucose by oxidation or fermentation, produced no acid in slant or butt of triple sugar iron agar, were sensitive to nalidixic acid (30- μ g disc), grew in *Brucella*

semi-solid broth at 42°C and 37°C but not at 25°C, grew in *Brucella* semi-solid broth containing 1% glycine but not in the semi-solid broth with 3.5% NaCl, and were hydrogen sulphide positive as detected by a lead acetate paper strip suspended over *Brucella* semi-solid broth containing 0.02% cysteine hydrochloride.¹⁸

RESULTS

The results of the faecal cultures of the four study groups are shown in Table I. *C. jejuni* was recovered from 3.8% and 4.3% of diarrhoeal patients from groups A and B, respectively. From the patients without diarrhoea, 2.6% of the children (group C) were positive for *C. jejuni* whereas none of the adults (group D) had this organism. In one case from group A, *C. jejuni* occurred together with *Salmonella* and in another case from the same group, *C. jejuni* occurred in association with enteropathogenic *E. coli*.

Other enteric pathogens, namely *Salmonella*, *Shigella* and enteropathogenic *E. coli* were isolated from 6.1%, 1.4% and 4.3% respectively, of the stools from the patients in group A. From group B, 4.3% and 1.5% of the patients had *Salmonella* and *V. parahaemolyticus*, respectively. *V. cholerae* was not detected from the stools of the patients in both the groups.

Out of the eight isolates of *C. jejuni* from group A, three were isolated from children below one year of age whereas the other five came from the one to ten

TABLE I
RESULTS OF FAECAL CULTURES

Group	No. tested	Number (%) positive for					Enteropathogenic <i>E. coli</i>
		<i>C. jejuni</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>V. cholerae</i>	<i>V. parahaemolyticus</i>	
A (children with diarrhoea)	212	8* (3.8)	13 (6.1)	3 (1.4)	0	0	9 (4.3)
B (adults with diarrhoea)	69	3 (4.3)	3 (4.3)	0	0	1 (1.5)	ND**
C (children without diarrhoea)	192	5 (2.6)	ND	ND	ND	ND	ND
D (adults without diarrhoea)	100	0	ND	ND	ND	ND	ND

* *Salmonella* and enteropathogenic *E. coli*, respectively, occurred together with *C. jejuni* in two cases from this group.

** ND denotes not done.

year age group. The three isolates from group B were from patients in the 20–40 year age group.

DISCUSSION

C. jejuni as a cause of diarrhoea has been reported with varying frequencies by workers in both developed and the developing countries.^{3,4,5} Our incidence of 3.8% and 4.3% in children and adults with diarrhoea, respectively, compares fairly closely with some of the studies done by investigators in the tropical countries. In Bangladesh, 5.2% of patients with clinical diarrhoea had *C. jejuni*.¹¹ In Indonesia, *C. jejuni* was the third most common enteric pathogen (5% of diarrhoeal stools) after *V. cholerae* (45%) and *Salmonella* (7%).¹⁵ In Saudi Arabia, however, only an incidence of 0.6% was obtained when 1,452 faecal specimens were examined.¹⁹

We found 2.6% of our children without diarrhoea carrying *C. jejuni* in their stools. An investigation conducted by De Mol *et al.*,²⁰ in Zaire revealed that 3% of their health control subjects had *C. jejuni* when they studied a total of 200 rural children. Incidences ranging from 0%⁷ to 16%⁸ have been found by other workers. None of our patients in the adult control group were carriers of *C. jejuni*. Similar findings were reported by Skirrow,² Brunton and Heggie,²¹ Steele and McDermott,²² Blaser *et al.*,²³ and Wright and Seager.²⁴ However, the sample size studied by us might be too small for any conclusive observation to be made.

Two of the cases in group A were infected by *C. jejuni* together with *Salmonella* and enteropathogenic *E. coli*, respectively, as the other enteric pathogen. Mixed infections with another enteric pathogen are common and have been observed by other investigators.^{2,10,12,14} Glass *et al.*,¹² found that mixed infection with another bacterial, viral, or protozoal pathogen occurred more frequently in patients with *C. jejuni* than in patients with rotavirus, *Shigella*, enterotoxigenic *E. coli* or *V. cholerae*.

Reports on the evaluation of transport media for *C. jejuni* in human faecal specimens are

available.^{25,26} In this study, *C. jejuni* was recovered from fresh stool specimens (ten isolates) and faecal samples in Cary-Blair transport medium (six isolates) in comparable numbers. Faecal specimens received in Cary-Blair transport medium were either processed immediately or kept at 4°C for up to 72 hr before culturing was done. This finding suggests that Cary-Blair medium can be successfully employed as an effective transport medium when immediate culturing of faecal specimens is not possible.

Our results showed that *C. jejuni* was the third most common enteric bacterial pathogen in children with diarrhoea, and its occurrence in adults was as frequent as *Salmonella*. Also, *Campylobacter* enteritis was shown to occur in patients of various age groups. This observation indicates that *C. jejuni* is one of the common bacterial causes of diarrhoea in Malaysia. Thus, this organism must be included as one of the bacterial pathogens by diagnostic laboratories in their routine examination of diarrhoeal stool specimens.

ACKNOWLEDGEMENTS

We thank Dr M.B. Skirrow of the Worcester Royal Infirmary, England, for confirming the identity of our first isolate of *C. jejuni*. The staff members of the Hospital Infection Control Unit, Department of Laboratory Services, General Hospital, Kuala Lumpur, and Miss A.S. Koay of the Bacteriology Division, Institute for Medical Research, gave technical assistance. We also thank Miss Y.P. Wong for typing the manuscript and the Director, Institute for Medical Research, for permission to publish this report.

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