

## **Campylobacter enteritis in Saudi Arabia**

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### SUMMARY

A 12-month survey on the incidence of campylobacter infection in 1217 patients with diarrhoea was carried out in Jeddah, Saudi Arabia. Campylobacters were isolated from 55 (4·5%) patients, second in prevalence to salmonellas (6·2%). Shigellas were isolated from 4·2% of patients. Campylobacter isolation rates were high in children of all ages, as well as in young adults (36·5% of all isolates were from adults aged 20–39 years). Isolation rates peaked in September and November. Analysis of the results showed that 69% were *Campylobacter jejuni* (mostly biotype IV) and 31% *C. coli*. Serogroups 5 and 23 (Penner scheme) and phage type 125 (Preston scheme) were most frequently isolated. Resistance to erythromycin and tetracycline was observed in 7·3 and 32·7% of the isolates. Campylobacters are an important cause of bacterial enteritis in Saudi Arabia, both in adults and in children, and should be sought routinely.

### INTRODUCTION

The genus *Campylobacter* has been known as both a pathogen and a commensal organism in domestic animals for some time. However, its association with acute diarrhoea and enteritis in man was recognized more recently by Butzler and co-workers in Belgium and by Skirrow in England [1, 2]. Campylobacter enteritis is now ubiquitous in many parts of the world. In the United Kingdom, surveillance reports from the Communicable Disease Surveillance Centre, Colindale (CDSC) for campylobacter infections increased from 9453 in 1980 to 32980 in 1989 [3] so that these now exceed those caused by all other bacterial species including salmonella. In other countries such as Norway, Romania, Yugoslavia, Hong Kong and India, *Campylobacter* species are the second most common cause of bacterial enteritis [4–8]. Campylobacter enteritis has been reported in the Kingdom of Saudi Arabia previously [9–11]. However, most laboratories in the Kingdom still do not routinely screen for campylobacter.

This report compares the prevalence of campylobacter infections with those of salmonella and shigella. The age and sex of patients and the seasonal distribution of *Campylobacter* species and their antibiotic sensitivity patterns were investigated. For the first time in the Kingdom the serogroups, biotypes and phage types of campylobacter isolates were determined.

## SUBJECTS AND METHODS

*Population studied*

The population studied comprised all patients with acute diarrhoea (i.e. those with diarrhoea, diarrhoea and vomiting or abdominal pain) attending the National Guard King Khalid Hospital (NGKKH) for a period of one year (May 1989–April 1990).

Patients attending NGKKH were mainly employees of the Saudi Arabian National Guard or their dependents. Other patients included those referred from other hospitals and NGKKH employees. Therefore, NGKKH is not one of the general hospitals in Jeddah and serves mainly a community within the city. The age of each patient submitting a sample was recorded. For patients found to be infected with campylobacter, their sex, nationality and clinical symptoms were also recorded. Multiple specimens from patients on any one day and from patients whose first specimen was positive were excluded.

*Laboratory methods**Salmonella and shigella*

Samples were cultured for salmonellas and shigellas on xylene lysine desoxycholate agar, desoxycholate citrate agar and in selenite enrichment broth. API 20E test strips (API Diagnostic Laboratories, 69280 Marcy-l'Étoile, France) and specific salmonella and shigella antisera (Wellcome Diagnostics, Dartford, England) were used for biochemical and serological identification.

*Campylobacter*

A 10  $\mu$ l loopful of the specimen was inoculated into Preston campylobacter selective enrichment broth (Oxoid CM67, SR117 and SR84 Oxoid Ltd, Basingstoke, UK); bottles were filled to the top to ensure microaerobic conditions. After incubation for 24 h at 42 °C, a loopful of the suspension was sub-cultured on modified CCDA–Preston blood-free campylobacter selective medium (Oxoid CM739, with SR125). Earlier work at NGKKH indicated that this combination of broth and agar was best for the isolation of *Campylobacter* species (Zaman, unpublished data). The CCDA plates were incubated at 42 °C micro-aerophilically for 48 h using BBL Gaspaks and jars. A campylobacter control strain was sub-cultured and incubated with each batch of test plates to ensure that the conditions were suitable for isolation of the pathogen. All colonies which morphologically resembled *Campylobacter* species were Gram-stained and subjected to an oxidase test. Campylobacter isolates were sub-cultured on Columbia Blood Agar (CBA) supplied by Saudi Prepared Media for sensitivity testing using tetracycline (Te10  $\mu$ g), erythromycin (Em10  $\mu$ g) and gentamicin (Gm10  $\mu$ g). Resistance was recorded when the zone size was reduced; a fully sensitive 'wild-type' strain (zone size for Te > 18 mm, Em > 18 mm, Gm > 15 mm) was used with each batch of tests. All isolates were stored at 4 °C on Amies transport medium swabs with charcoal for serogrouping, biotyping and phage-typing tests. The positive isolates were sub-cultured every 2 weeks on CBA and fresh transport medium swabs were seeded to maintain viability of the organisms.

### *Biotyping*

Tests for rapid hippurate hydrolysis, rapid hydrogen sulphide production and deoxyribose nucleic acid hydrolysis (DNase) as described by Lior [12] were used for biotyping all campylobacter isolates at NGKKH.

### *Serogrouping*

All serogrouping work was done by PHLS, Withington Hospital, Manchester, UK. The Penner scheme was used for serogrouping.

### *Phage typing*

This work was carried out by PHLS, Royal Preston Hospital, Preston, UK.

## RESULTS

Faecal specimens from 1217 patients were cultured during a one-year period. The number and percentage of each pathogen isolated are shown in Table 1, and seasonal distributions in Fig. 1. It will be observed that all three pathogens were isolated most often during the last quarter of the year. More than one pathogen was isolated from 10 patients; campylobacter and salmonella in 9 patients; campylobacter and shigella in 1 patient. *Salmonella enteritidis* and *S. typhimurium* were the most prevalent salmonellas; *S. typhi* and *S. paratyphi* were not isolated. The shigellas included *Shigella sonnei* (40%), *Sh. boydii* (31%) and *Sh. flexneri* (29%). *Sh. dysenteriae* was not isolated.

### *Campylobacter-infected patients*

Among the 55 patients infected with *Campylobacter* species, there were 34 Saudi and 20 non-Saudi patients. Non-Saudi patients included Europeans (EU) (9), Yemeni (YE) (5), Indian subcontinentals (ISC) (3) and other Middle Eastern (ME) nationalities (3). Twenty-nine were male and 26 female (M:F ratio 1.1:1). The age distribution of infection is shown in Table 2. This indicates the highest rates are observed in children under the age of 10 years and in young adults.

### *Clinical features*

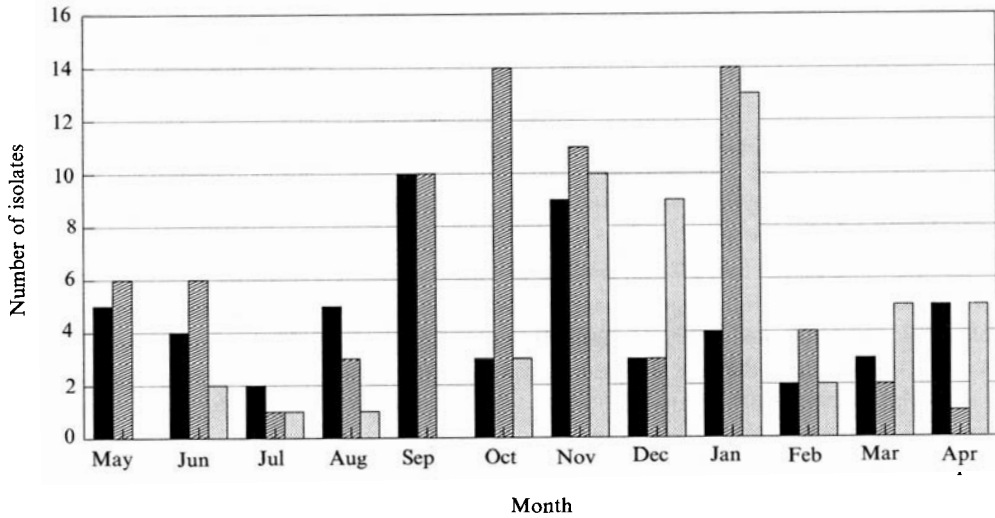
Diarrhoea alone was observed in 13/55 (23.6%) cases; diarrhoea and vomiting were observed in 11/55 (20%) cases and 7/55 (12.7%) patients had pyrexia in addition to diarrhoea and vomiting. In 3/55 (5.5%) cases, blood and mucus was present in the faeces. In one case the infection was systemic, the organism being isolated from the blood of the patient. In another 4 patients there were other underlying factors such as Hodgkin's disease (2 Yemeni nationals, 1 fatal), leukaemia (1 Saudi patient) and Behçet's disease (1 Saudi patient). Acute abdominal discomfort only was described in 10 cases. Frequency of acute attacks at 30–40 times a day and episodes lasting for about 2 weeks were recorded. Most cases were self-limiting, but in three cases erythromycin was administered.

### *Campylobacter isolates*

Of 55 isolates tested, 38 (69%) were *C. jejuni* and 17 (31%) were *C. coli*. Among the *C. jejuni* isolates, biotype IV predominated (Table 3). The distribution of

Table 1. *The number and percentage of bacterial pathogens at NGKKH*

	Isolates (%)
<i>Campylobacter</i> sp	55 (4.6)
<i>Salmonella</i> sp	75 (6.7)
<i>Shigella</i> sp	51 (4.2)

Fig. 1. Seasonal distribution of enteric pathogens. ■, *Campylobacter* sp.; ▨, *Salmonella* sp.; □, *Shigella* sp.Table 2. *The age distribution of campylobacter infections*

	Age in years										
	< 1	1-4	5-9	10-14	15-19	20-24	25-29	30-34	35-39	40-44	> 45
# P	11	8	7	2	2	2	12	3	3	—	5
T P	141	175	73	42	34	77	134	124	99	60	258
% P	7.8	4.5	9.5	4.8	5.9	2.6	9.0	2.4	3.0	0	1.9

# P, Number of positive patients; % P, percentage of positive patients; T P, total number of patients in the age group.

Table 3. *Biotypes of campylobacters isolated*

Biotype	Isolated (%)
<i>C. jejuni</i> IV	20 (36.4)
<i>C. coli</i> I	12 (21.8)
<i>C. jejuni</i> II	11 (20.0)
<i>C. jejuni</i> I	5 (9.1)
<i>C. coli</i> II	5 (9.1)
<i>C. jejuni</i> III	2 (3.6)
Total	55 (100)

Penner serogroups in the 50 isolates tested is shown in Table 4. Serogroups 5 and 23 were the most frequently represented. The phage-typing service became available only after the study had commenced, so only 26 isolates were typed. These results are shown in Table 5.

Table 4. *The serogroups of campylobacter isolated*

Penner serogroup	Isolated (%)
5	8 (16)
23	7 (14)
2	6 (12)
1	5 (10)
4, 13, 16, 50	3 (6)
9	2 (4)
9, 37	2 (4)
39	2 (4)
11	1 (2)
24	1 (2)
24, 49	1 (2)
Not typable	12 (24)
Total	50 (100)

Table 5. *The phage types of campylobacter isolated*

Phage type	Isolates (%)
125	8 (30.8)
69	3 (11.5)
121	3 (11.5)
91	2 (7.7)
116	1 (3.8)
84	1 (3.8)
52	1 (3.8)
55, 58	1 (3.8)
NT	6 (23.1)
Total	26 (100)

NT, Not typable.

Resistance to erythromycin was detected in 4 (7.3%) strains (3 of these were *C. coli*) and resistance to tetracycline in 18 (32.7%) isolates investigated. All isolates were sensitive to gentamicin.

#### DISCUSSION

The prevalence of campylobacter enteritis in Saudi Arabia has previously been documented [9–11]. The frequency with which campylobacters were isolated in these studies varied greatly. During this investigation campylobacter was isolated from 4.5% of patients. It was the second most prevalent pathogen, after *Salmonella* species (6.2%), and was followed closely by *Shigella* species (4.2%). Results in Yugoslavia were similar; the proportion of salmonellas was greatest and the numbers of campylobacters and shigellas were almost the same [6].

The analysis of the NGKKH campylobacter-infected patients by age, produced the most surprising findings. The high rates of infection in children were expected,

but not in young adults; 36.5% of all isolates were obtained from adults aged between 20 and 39 years (Table 2). This is similar to the pattern found in Europe and North America [13–15]. In most African, Middle Eastern and developing countries transmission is so high that children become immune by the time they are 5 years old, and clinically apparent infection is almost unknown in adults [8, 16].

The high incidence in young adults in the present study can be partly explained by the fact that some were expatriates. Thirteen of the 27 adult patients were non-Saudi, whereas only 7 of the 27 infected children under the age of 10 years were non-Saudi. It would be expected that the European expatriates would not have immunity and would therefore be susceptible to infection. The effects of a large non-indigenous population in the Kingdom on the prevalence of infectious diseases have been shown with other infections such as tuberculosis [17]. During this study only the nationality of patients who were positive for campylobacter was recorded; it was therefore not possible to calculate the proportion of SA and NSA patients in each age group.

Nevertheless, the fact that 13 Saudi adults were infected requires explanation. Possibly campylobacter transmission in Saudi Arabia is lower than in other Middle Eastern and African countries, so that many children do not become immune. Alternatively, immunity to campylobacter infection might wane with a reduction of transmission brought about by rapid social advancement and improved hygiene.

The seasonal distribution of campylobacter enteritis in Saudi Arabia has not been documented before. During this survey campylobacter infections peaked in September and November. This period is mostly dry and temperatures are not at their peak (the hottest months are July and August). The seasonal distribution of infection varies geographically. In temperate zones, incidence is highest in summer, whereas in tropical zones there is no obvious variation although it appears to be higher in rainy seasons. It is recognized that the numbers reported here are small and the seasonal patterns in Saudi Arabia need to be established by further studies over longer periods.

*C. coli* accounted for 31% of campylobacter infections which is higher than usual. Other places with reported high proportion of *C. coli* infections are Yugoslavia [6], Hong Kong [7], Chile [18] and the Central African Republic [19]. The high proportion of *C. jejuni* biotype IV (rapid H<sub>2</sub>S and DNase tests positive) has not been reported elsewhere.

Strains of serogroups 5 and 23 were isolated most often in this study. These are less prevalent elsewhere, although strains of serogroup 5 are common in the Central African Republic [19]. There are other examples of differences in strain distribution. In Bangladesh serogroups 53 and 15 are most prevalent [16], but these were not encountered during this study. Strains of the serogroup 4, 13, 16, 50 complex were the most frequent in Europe [20], but they accounted for only 6% of isolates in this present series. Comments on phage-type distribution cannot be made as so few studies have included phage typing in their protocols.

The erythromycin resistance rate of 7.3% found in this study is much the same as the rates found in Europe and North America. Far higher rates have been reported from some developing countries.

This study has shown that campylobacters are the second most frequent cause of acute bacterial enteritis in Saudi Arabia, affecting adults as well as children. The case for laboratories to take appropriate measures to isolate these organisms has been made in the past [21] and these results emphasize this need, both for epidemiological and clinical purposes. The modified blood-free agar used in this study, which is easy to prepare and extremely effective, is recommended for routine use.

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