# ISOLATION AND IDENTIFICATION OF THERMOPHILIC CAMPYLOBACTERS FROM DIARRHOEAL CHILDREN IN BAGHDAD

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*SUMMARY: Thermophilic campylobacters were isolated at "Saddam's Central Hospital for Children" from the stool of 120 children having diarrhea. Specimens were watery or bloody mucous. Twelve isolates were identified according to colonial morphology, Gram stain, motility, oxidase, catalase, biochemical tests and cell morphology using light and electron microscopes. The isolation percentage was 10%.*

*Key Words : Campylobacter, children, diarrhea.*

## INTRODUCTION

*Campylobacter* is recently recognized as an important etiological agent of bacterial enteritis (12). Campylo- bacter enteritis was first reported at the end of the nineteenth century by Theodor Escherich. He discovered, in the feces of children with diarrhea, spiral organisms that could not be cultured on solid media (8). King in 1957 was the first to recognize that these organisms could be associated with enteric diseases (7).

During the 1980s major advances in the isolation of this organism were obtained including the development of isolation technique. Following this progress it became obvious that the incidence of *C. jejuni* enteritis exceeds that of the human enteric pathogens such as *Salmonella Spp.* (4). Reports of enteritis caused by *C. jejuni* and the very similar organism *C. coli* have risen in the developing as well as developed countries (1,3,14).

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## MATERIALS AND METHODS

Stool specimens were collected from 120 patients admitted to Saddam's Central Hospital for Children in the period from late September to the end of October 1991, suffering from diarrhea (which was watery or bloody mucous). The ages of the patients ranged from 2 months to 7 years.

The specimens were streaked after 2-6 hours after collection on Skirrow, Butzler or Preston selective media (Oxoid). The plates were then incubated for 24-48 hours at 42°C using gas generating kits (Oxoid) to supply microaerophilic conditions (85% nitrogen, 10% carbon dioxide and 5:oxygen). The identification depended on colonial morphology, Gram stain, oxidase, catalase, biochemical tests and cell morphology using light and electron microscopes according to Pead's method (11).

## RESULTS AND DISCUSSION

### Morphology

Twelve isolates were identified positively as being Campylobacters. Two types of non hemolytic colonies were found in the primary isolation, the first was watery

Figure 1: Campylobacter isolate 65 grown on blood agar for 24 h at 42°C. Two types of colonies were observed small translucent and large gray.

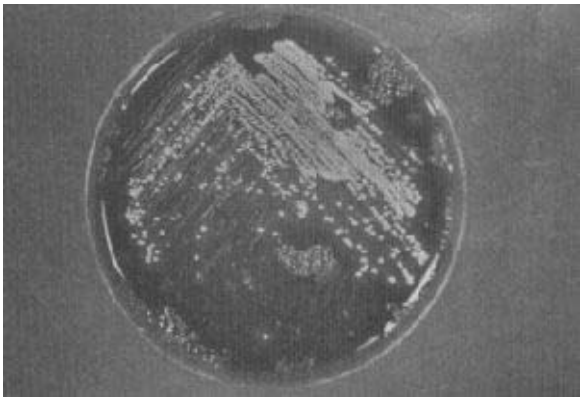
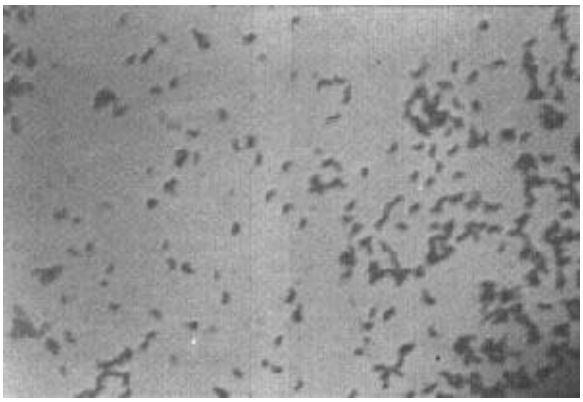


Figure 2: *Campylobacter* smear taken from 24 h growth of isolate 44 on blood agar colored with carbol fuchsine. Comma, spiral and sea gull wing shapes are seen (magnified 1200x).



small (1-2 mm in diameter) and translucent and the second was large, flat and gray in color (Figure 1). Bacterial cells were gram negative with spiral, comma, gull wings and S-shapes (Figure 2). Coccoid forms were found in 72 hour cultures (Figure 3).

Electron microscope showed the presence of amphitrichous flagellation with one flagellum at each pole (Figure 4). Spiral, comma and s-shape *Campylobacter*s were all very clear. Some cells joined each other in chains (Figures 5, 6, 7,and 8). Pleomorphism and coccoid bodies of *Campylobacter* were observed by many workers (2,12). The spiral shape of *Campylobacter* was used as a characteristic feature to differentiate this organism from other bacteria (6).

**Biochemical tests**

All isolates were negative for gelatinize, lipase, indole production and carbohydrate utilization. All isolates were positive for oxidase and catalase. Twenty five percent of the isolates were *C. coli* 75% were *C. jejuni* according to the hippurate test. It was found that the hippurate test is the best for the differentiation

Figure 3: Coccoid forms of *Campylobacter* colored with carbol fuchsine. Spiral and sea-gull wing shapes are seen. Smear was taken from 72 h growth of isolate 44 on blood agar at 42°C (Magnification 2000x).

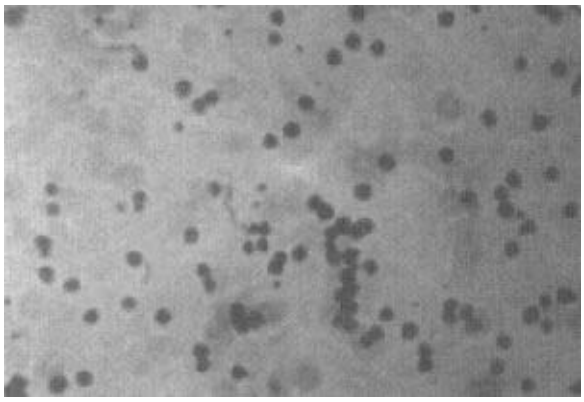


Figure 4: Electron microscope photograph of *Campylobacter* isolate 44 grown on blood agar for 24 h at 42°C. Spiral form with one flagellum at each pole of *Campylobacter* isolate (E. M. photograph magnified 10000x).

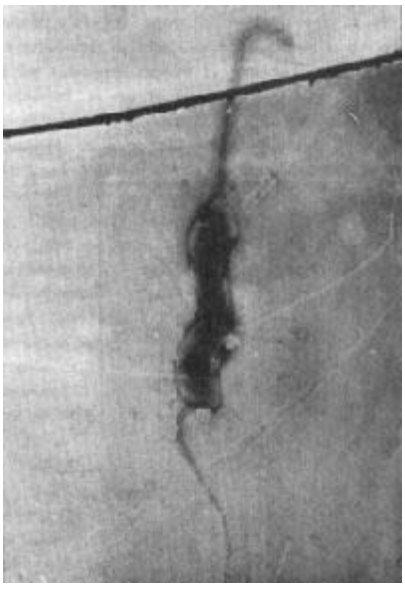
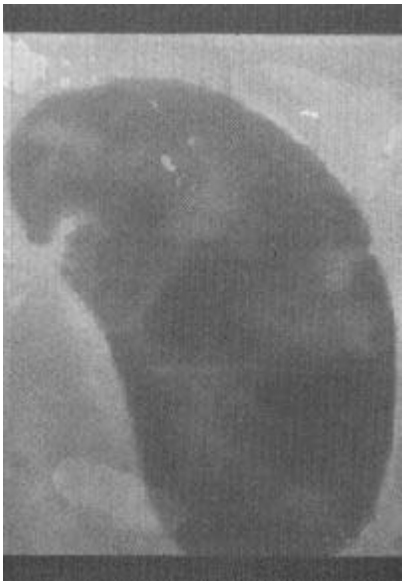


Figure 5: *Campylobacter* isolate 44 grown on blood agar for 24 h at 42°C. Spiral form is seen (E.M. photograph magnified 10000x).

Figure 6: *Campylobacter* isolate 44 grown on blood agar for 24 h at 42°C. S-shape form is seen (E. M. photograph magnified x 46000x)

Figure 7: *Campylobacter* isolate 44 grown on blood agar for 24 h at 42°C. Comma shape is seen (E. M. photo magnified 46000x).



between the two thermophilic species *C. jejuni* and *C. coli* (5,9).

**Growth at 25°C and 42°C**

All isolates grew very well at 42°C but not at 25°C. This test is used to identify thermophilic group of campylobacters (13).

**Percentage of isolation**

The percentage of isolation of thermophilic *campylobacter*s from stool specimens of children was 10% in comparison to the 7 isolation percentage which was found by Makkia *et. al.* in 1988 (10).

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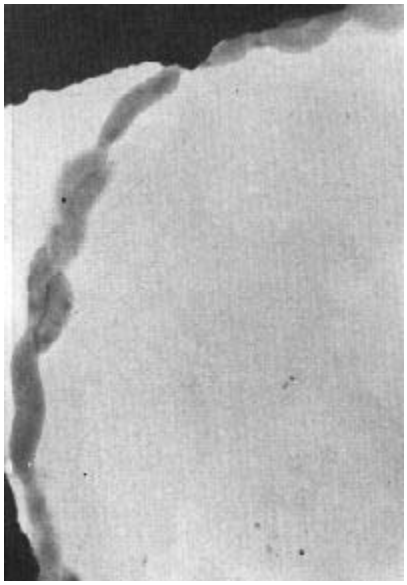
Figure 8: *Campylobacter* isolate 44 grown on blood agar for 24 h at 42°C, different cell shapes arranged in long chain. (E.M. Photomagnified 10500x).



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