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Coagglutination test for rapid noncultural diagnosis of human Campylobacteriosis

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Abstract. – **Forty diarrhoeic stool samples of domestic animal handlers in a rural area of India were cultured and screened for detection of *Campylobacter jejuni* by Co-agglutination technique (CoA). Seven *C. jejuni* strains were isolated by culture and 6 faecal samples gave positive CoA test. The specificity and sensitivity for negative and positive culture were 96.9% and 85.7% respectively. This is first report of detection of *Campylobacter* enteritis of human beings using CoA technique directly from stool samples.**

Key Words:

Coagglutination test, Rapid diagnosis, Campylobacter jejuni.

Introduction

The agent *Campylobacter* has been identified as a common agent causing enterocolitis in man[1,2]. These microorganisms are also present in the feces of domestic animals like cattle, sheep, pigs and chicken which act as reservoir for *Campylobacter* producing human Campylobacteriosis[3,4]. Chicken have been found to be the commonest reservoir of *C. jejuni*[5]. In India, rearing of these domestic animals is a very common practice particularly in the rural areas. The data relating to the occurrences of human Campylobactoriosis is sparse in India[6]. Again, the procedure for isolation and identification of Campylobacter Spp is relatively difficult[7] and require special attention for culture of the organism[8]. Although some non-cultural methods have been developed but they are technically difficult and also costly[9,10]. The present study was therefore, planned to develop a non-cultural technique for identification of *C. jejuni* using Co-agglutination (CoA) method.

Materials and Methods

The study area from where the samples were collected is situated 35 Km away from the city and rural in nature. Domestic animal rearing is a common practice in majority of the population and the principal mode of earning is agriculture. A total of 40 diarrhoeic faecal samples from domestic animal handlers were collected for this study. Spot inoculation of the faecal matter was done onto Butzler's selective media with antibiotic supplement and incubated immediately in a candle jar microaerophically[11]. The remaining faecal samples from where culture was done, were transferred to a sterile container having 5 ml of sterile Phosphate Buffer Saline (PBS) at pH 7.2 using sterile technique. The samples were labelled properly and sent to the laboratory for processing. The candle jars were incubated at 42 deg C for 48h for selective isolation of *C. jejuni*. The PBS suspensions were vortexed and then centrifused at 3000 RPM for 10 mins. The supernatant were collected aseptically and kept in a refrigerator for further processing. The Butzler's plates were examined after 48h for the presence of characteristic colonies of *C. jejuni* which was subsequently identified using standard technique[12].

C. jejuni strain maintained in the laboratory was purified and suspended in PBS (pH-7.2). The suspension was kept in vaccine bath at 60 deg C for 1hr. The opacity of the suspension was adjusted to 10 to the power 9 organisms per ml using McFarland tube. Adult male rabbits weighing approximately 1000 g were used for immunization. Preparation of antisera and CoA test using Staphylococcus aureus Cowan I strain were followed according to the method described by Latitha et. al[13].

Table I. Comparison of results of CoA and culture of *Campylobacter jejuni*.

Test results for CoA (No.)	*Culture of C. jejuni positive (No.)*	*Culture of C. jejuni negative (No.)*
Positive (7)	6	1
Negative (33)	1	32
Total (40)	7	33

Sensitivity: 6 of 7 (85.7%) for positive *C. jejuni* culture. Specificity: 32 of 33 (96.9%) for negative *C. jejuni* culture. Predictive value for positive CoA test results 6 of 7 (85.7%). Predictive value for negative CoA test results 32 of 33 (96.9%).

Results

Out of 40 diarrhoeic samples cultured on selective media 7 (17.5%) yielded growth of Campylobacter Spp and all were subsequently identified as *C. jejuni* by CoA method, 6 out of 40 faecal suspensions gave positive agglutination reaction (Table I). Sensitivity and specificity of CoA for positive and negative cultures were 85.7% and 96.9% respectively. Predictive value for positive CoA and negative CoA were 85.7% and 96.9% respectively.

Discussion

Non cultural rapid screening of faecal samples for bacterial pathogens is an useful tool for etiological diagnosis. CoA test have been employed for such diagnosis in many diseases like Salmonella[14], Brucella[15] and Vibrio cholerae[16], CoA was not employed earlier for diagnosis of Campylobacter infection.

Campylobacter Spp require a complex media and atmosphere for growth which is not feasible in most of the laboratories particularly in a developing country like India. Campylobacter Spp are now among the commonest identified causes of enteritis in man in developed country[17]. Domestic animals particularly the chicken are the principal source of infection. In India, particularly in the rural areas, rearing of domestic animals and farming of poultry are seen commonly among the rural people which also provide some income to them. In most of the areas of study proper sanitary measures and hygiene are not maintained. Campylobacteriosis is often a self-limiting disease and does not require antibiotics for treatment in immunocompetent individuals. Again, in absence of available cultural methods in most of the laboratories and also in hospitals, many cases of Campylobacter enteritis remain undetected. Therefore, rapid and inexpensive non-cultural method would be of much help in the diagnosis of human Campylobacteriosis. Recently, PCR and a commercial ELISA for direct detection of Campylobacter from stool samples have been developed[18] but availability and utilisation of these tests for diagnosis of Campylobacteriosis remain a costly procedure. In this study CoA shows a high degree of specificity and the test does require any special training of laboratory personnels and the reagents also can be kept for many months keeping in a refrigerator[19]. In the light of high degree of specificity it is recommended to use CoA on stool samples directly for quick and specific diagnosis of human Campylobacter enterities.

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