## *Original Articles*

# CLOSTRIDIUM DIFFICILE IN ANTIBIOTIC ASSOCIATED PEDIATRIC DIARRHEA

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**ABSTRACT**

*A case control study was carried out at the medical wards of Dr. B. C. Roy Memorial Hospital for Children, Calcutta, between January and September 1989. One hundred eleven hospitalized children up to the age of 5years, receiving antibiotics for different medical problems, developed antibiotic associated diarrhea. Isolation of Clostridium difficile as sole pathogen was very low (3.6%) from these patients. Fecal samples of 111 case matched control children were also screened for C.difficile. Only 2.7% fecal samples of control children were positive for C. difficile. All the strains of C. difficile isolated from antibiotic associated diarrhea cases showed neutralisable cytotadn in in vitro test. In contrast none of the strains isolated from control children showed cytotaxicity. This study suggests that C.difficile is not an important pathogen related to antibiotic associated diarrhea in children at this hospital.*

**Keywords:** *Clostridium difficile, Antibiotic associated, Diarrhea.*

*Clostridium difficile* has been implicated as a causative agent of antibiotic-associated diarrhea, colitis and pseudomembranous colitis(l-3). Antibiotic exposure and admittance to hospital are the known risk factors for *C.difficile* diarrhea and colitis(4). In the developed countries, *C.difficile* has recently become recognized as an important pathogen causing antibiotic associated diarrhea in adults(5). Among the pediatric age group, the symptomatic carriage of *C.difficile* is common but the incidence of C. *difficile* associated diarrhea or colitis is rare(8). However, information regarding the incidence of *C.difficile* from antibiotic associated diarrhea among children in the developing countries is still meager. This report presents the results of isolation of *C.difficile* from antibiotic associated diarrhea, in children at a pediatric hospital in Calcutta.

## Patients and Methods

A hospital based study was carried out at the medical wards of Dr. B.C. Roy Memorial Hospital for Children, Calcutta, between January and September 1989. Hospitalized children up to the age of 5 years, suffering from different medical problems (other than diarrhea) and treated with antibiotics were followed up. Physi-

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cians searched for antibiotic associated diarrhea cases in their daily hospital ward round. If patients developed antibiotic associated dicxrhea during follow up period, special records were made about age, sex, present and final diagnosis, antibiotic administered, frequency of stools per day, stool character *(e.g.,* watery, blood and mucus or mucoid), abdominal pain, tenesmus, fever (>38°C) and of hydration status. *C.difficile* associated diarrhea cases were treated with metronidazole at the dose of 40 mg/kgbody weight/day for 10 days even after cessation of diarrhea. They also received oral rehydration therapy for correction of dehydration. Patients were discharged from the hospital after passing the first formed stool if they were cured from original illness. At the time of discharge parents of the children of both cases and controls from whom *Cdifficile* was isolated, were advised to bring their children for follow up weekly for 2 months. They were also advised to contact the investigators immediately if their children developed diarrhea, at the time of follow up. Stool samples of these children were collected to screen for *C.difficile.*

Antibiotic associated diarrhea was defined in those cases who developed diarrhea more than 72 hours after hospitalization and received antibiotics before development of diarrhea. Diarrhea was defined if patients passed a minimum of 4 unformed stools within a 24 hour period.

*Controls: A* case matched control group was selected from hospitalized patients without diarrhea who had the following characteristics: located on the same hospital ward as a case, hospitalized within two weeks of a case, having the same or similar underlying medical diagnosis and treatment.

*Sample collection and bacteriological methods:* Fecal samples were collected from antibiotic associated diarrhea cases in the sterile MacCarteny's bottles by sterile rectal catheters. Mothers of control children were advised to submit freshly passed stool samples of their children in sterile bottles. Fecal samples were processed immediately in the laboratory on to a selective medium, cycloserinefoxitin-fructose agar (CCFA)(7). Inoculated plates were incubated in an anaerobic jar with gas generating kit (Oxoid) at 37°C and examined after 48 hours for presence of *C.difficile.* Anaerobiosis was monitored by resazurin indicator strips (Oxoid). If the colonies of *C.difficile* were not found, the plates were reincubated for a further 48 hours and visually screened for *C.difficile* before being discarded. Colonies of distinctive morphology were Gram stained and subcultured in Robertson's cooked meat medium. After 3 days incubation, the cultures were checked for purity and identified by biochemical reactions to glucose (+ve), frftctose (+ve), mannose (+ve), lactose (—ve) and sucrose (—ve) as recommended by the anaerobic laboratory manual(8). Fecal samples were also processed for isolation of olher bacterial enteropathogens using standard techniques(9). *In vitro* cytotoxin production of an isolated of *Cdifficile* was determined by growing it anaerobically in brain-heart infusion broth (Difco Laboratories, Detroit, Michigan) for 48 to 72 hours at 37°C. Culture filtrate was assayed for its cytotoxicity on VERO tissue culture monolayer(lO). Cytotoxicity of all the fecal samples were also assayed on VERO cells(10). *C.difficile* cytotoxin activity was confirmed by neutralization of the cytopathic effect by using specific *C.difficile* antitoxin (VIP Anaerobe Laboratory, Blacksburg, VA).

## Results

One hundred eleven hospitalized children aged up to 5 years suffering from

different medical problems, receiving antibiotics, developed diarrhea during the period of observation. Fecal samples of these children were screened for *C.difficile. C.difficile* was isolated in 4 (3.6%) cases as sole entero-pathogen. No other clostridia was isolated. All these strains demonstrated neutralizable cytotoxicity in *in vitro* test.

Similarly, stool specimens of 111 case matched control children who fulfilled the study protocol were also analysed. *C.difficile* was isolated from 3 (2.7%) of these controls. However, none of the strains isolated nfrom control children showed
cytotoxicity. Extracts of all the fecal samples (both from cases and controls) did not show cytotoxicity.

*Shigella* species, *Salmonella typhimurium,* Enteropathogenic *E. coli,* enterotoxigenic *E. coli* were isolated from 13.5%, 9.0%, 8.1%, 1.8% cases and 7.2%, 6.3%, 6.3%, 0.9% control population, respectively as sole pathogen.

All the *C.difficile* associated diarrhea patients had history of watery diarrhea, abdominal pain and fever (>39°C). All the children were aged between 7 and 24 months from whom *C.difficile* was isolated. Patients developed *C.difficile* associated diarrhea on an average 16.5 (±SD, 2.5) days after hospitalization. Mean duration of hospitalization of *C.difficile* associated control children was 16.8 (± SD, 3.2) days at the time of collection of sample. *Table I* shows the age group of antibiotic associated diarrhea cases and their controls. Antibiotic used in cases and control children is shown in *Table II.* Cases and their controls received similar type of antibiotics. Cytotoxin producing *C.difficile* was isolated from antibiotic associated diarrhea cases who received ampidllin, cephalosporin or gentamicin either singly or in combination. All the children suffering from *C.difficile* associated diarrhea were treated with metronidazole and the symptoms resolved within 5 days after initiation of therapy. *C.difficile* could not be isolated from stool samples collected from all the children attended for follow up. Cases

TABLE 1- *Age Distribution of Antibiotic Associated Dianne a Cases and their Hospitalized Controls who we Sened for C.difJicile*

| Age groups (mo) | No. of nosocomial diarrhea cases | | No. of hospitalized controls | |
|---|---|---|---|---|
| | Screened | Positive for *C.difficile* | Screened | Positive for *C.difficile* |
| 0-6 | 13 | 0 | 11 | 0 |
| 7-12 | 16 | 1 | 15 | 1 |
| 13-24 | 28 | 3 | 30 | 2 |
| 2S-J6 | 22 | 0 | 23 | 0 |
| 37-48 | 14 | 0 | 14 | 0 |
| 49-60 | 18 | 0 | 18 | 0 |
| Total | 111 | 4 | 111 | 3 |

**TABLE** II- *Antimicrobial Agents Used and Isolation of C.difficile from Fecal Samples of Antibiotic Associated Diarrhea Patients and Controls*

| Antimicrobial agents used | Nosocomial Diarrhea Cases | Nosocomial diarrhea cases positive for *C.difficile* | Control patients | Control patients positive for *C.difficile* |
|---|---|---|---|---|
| Ampicillin | 12 | 1 | 13 | - |
| Amoxycillin | 10 | - | 9 | - |
| Cloxacillin | 10 | - | 11 | - |
| Cephalosporin | 8 | 1 | 10 | - |
| Amikacin | 5 | - | 4 | - |
| Gentamycin | 12 | 1 | 13 | 1 |
| Metronidazole | 4 | - | 5 | - |
| Erythromycin | 4 | - | 5 | - |
| Trimethoprim-sulphamethoxazole | 9 | - | 10 | - |
| Chloramphenicol | 5 | - | 6 | - |
| Antimicrobial agents in combination | 32 | 1 | 25 | 2 |
| Total | 111 | 4 | 111 | 3 |

and controls did not suffer from diarrhea during follow up period.,

## Discussion

This .hospital based case control study showed low isolation of *C.difficile* from both antibiotic associated diarrhea cases (3.6%) and their controls (2.6%). There is no difference in the rate of isolation of *C.difficile* in these two groups which suggests that *C.difficile* is not an important pathogen related to antibiotic associated diarrhea in children at this hospital. Similar results have been reported from studies conducted in developed countries(6). Ayyagiri and colleagues have also reported positive culture for *C.difficile* in 2 (8.3%) of 24 antibiotic recipients in pediatric age group(11). However, association of *C.difficile* or its cyto-toxin in 9% to 60% antibiotic associated diarrhea in adults were observed in different studies from developed countries(12,13). It has been documented that any antibiotic or chemotherapeutic agent can produce *Cdifficile* associated diarrhea but the disease is seen most commonly with the use of clinda-mycin(2). In some parts of the developed countries, clindamycin alone is responsible for *C.difficile* associated diarrhea in 10% to 25% of hospitalized patients(14). It has also been documented that ampicillin, aminoglycosides and cephalosporins are also common antibiotics which produce *C.difficile* associated diat-

rheas(13,15). Bartlett also reported that 5-10% of patients develop *C.difficile* associated diarrhea after receiving ampicillin alone(14). In India clindamycin is not marketed but ampicillin, cephalosporin and amnoglycoside are used frequently either singly or in combination for the treatment of various infections in pediatric patients. Inspite of frequent use of these antibiotics, detection rate of *C.difficile* from antibiotic associated diarrhea in children is very low in the present study as compared to that of the rate of detection in developed countries(6). In contrast, studies from this hospital showed that cytotoxin producing *C.difficile* could be isolated from 8.4% to 11.1% of hospitalized diarrheal children who acquired the infection from the community(16,17). Gupta and Jadav from North India also reported 25.3% isolation of *C.difficile* from diarrheal patients of all age groups(18). Although all the strains of *C.difficile* strains were isolated from patients of 7 to 24 months age group, no significance could be attributed to this finding as only a small number of patients were positive for *C.difficile*.

In the present study none of the fecal extracts showed cytotoxicity. *In vitro* findings showed that *C.difficile* cytotoxin is inactivated by the myeloperoxidase system of neutrophils and $H_2O_2$ from lactobacillus acidophillus(19), may explain the absence of cytotoxin in fecal samples though toxin producing *C.difficile* was isolated from the same specimen.

Vancomycin is the drug of choice for the treatment of *C.difficile* associated diarrhea. Response rates of vancomycin have ranged from 95% to 100% in most of the studies(14,15). Unfortunately, vancomycin is not available in India. The alternative antimicrobial agent most often used to treat this infection is metronidazole(20). Our patients also responded well to metronidazole therapy and did not have relapse within 2 months of follow up. It has also been observed that symptom free excretors of *C.difficile* control children did not suffer from diarrhea subsequently.

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