

A STUDY ON PREVALENCE OF BACTERIAL DIARRHEA AND ITS AGENTS

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Abstract

Prevalence of etiologic agents associated with diarrhea was carried out. Of over 200 cases, 46 cases were found infected. 31 (67.39%) cases were positive for *Vibrio cholerae*, from remaining cases *Vibrio parcolans*, *Vibrio neocistes*, *E. coli*, *E. freundii*, *Proteus inconstans* and *Alkaligenes faecalis* were isolated. Proteolytic clostridia were also isolated from 25 cases. The toxin of these clostridia digested the muscles at the injection site. The prevalence of micro-organisms after recovery was also significant and of academic interest (JPMA 29:239, 1979).

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Introduction

Worldwide sporadic cases of gastroenteritis produce health hazards. *Shigella*, *Staphylococci*, *Giardia lamblia*, *Entamoeba histolytica*, enteropathogenic serotypes of *Escherichia coli* and enteroviruses usually account as conventional pathogens for the disease (Gorbach et al., 1975; Krupp & Chatton, 1976; Bennington et al., 1972). Besides, cholera exists endemically in Asiatic countries (Cruickshank 1968).

The epidemic of cholera has been controlled through proper application of quarantine regulations and control measures and nowadays it is confined to few foci in India, Pakistan and other Asiatic countries. In spite of empirical application of control measures it still exists endemically in Bengal Basin, with the Deltas of Ganges and Brahmaputra, throughout the year (Cruickshank 1968; Dubos 1958). The reason may be the low living standard, mal-nutrition and insanitary conditions. In Pakistan, the infection flares up occasionally, particularly during rainy season. The present study was undertaken during such outbreak to incriminate the agents responsible for such diarrheas and evaluate the normal flora after such syndrome.

Material and Methods

Blood agar, alkaline blood agar, alkaline taurocholate peptone broth, alkaline taurocholate agar, Mac-Conkey's agar, E.M.B. agar, Nutrient agar, T.S.I. medium were used (Difco).

Glucose, maltose, sucrose, lactose, xylose, fructose, galactose, mannitol, dextrin, mannose, dulcitol, rhamnose, inositol, inulin, S. citrate, gelatin were employed for biochemical and fermentation reactions. Indole, MR, VP, NR, Cholera red test were also performed.

Healthy rabbits (1500 gms or more were injected intramuscularly for determination of toxigenicity of the isolates as follows :

- 1) 2.0 ml of 24 hours broth (enriched with horse serum) culture of *Cl. histolyticum*.
- 2) 2.0 ml of *Vibrio cholerae* broth culture.
- 3) 1.0 ml of *Cl. histolyticum* broth culture + 1.0 ml of *Vibrio cholerae* broth culture.
- 4) 1.0 ml of *Cl. histolyticum* broth culture + 1.0 ml of *Proteus mirabilis* broth culture.

Table I: Distribution of Etiologic Agent Associated with Diarrheal Disease with Respect to Age

Age (in years)	No. of Infected Patients	Aerobic micro-organisms									
		<i>Vibrio cholerae</i>	<i>Vibrio parvulus</i>	<i>Vibrio nectans</i>	<i>Escherichia coli</i>	<i>Escherichia freundii</i>	<i>Proteus mirabilis</i>	<i>Alkaligenes faecalis</i>	<i>Clostridium histolyticum</i>	<i>Clostridium paraputrificans</i>	<i>C. histolyticum and C. paraputrificans</i>
1-10	14	11	—	1	2	—	—	1	5	2	3
11-20	8	5	1	—	—	1	—	—	3	—	1
21-30	9	4	—	—	1	1	—	1	2	2	3
31-40	6	2	1	—	1	—	—	1	1	1	3
41-50	5	3	2	—	—	—	—	—	—	2	2
51-60	2	2	—	—	—	—	—	—	1	—	1
61-70	1	1	—	—	—	—	—	—	—	—	—
71-80	1	1	—	—	—	—	—	—	—	—	—
Total	46	31	4	1	4	2	1	3	12	7	13

Table II: Distribution of Etiologic Agent Associated with Diarrheal Disease with Respect to Sex

Patients	No. of infected cases	Aerobic micro-organisms									
		<i>Vibrio cholerae</i>	<i>Vibrio parvulus</i>	<i>Vibrio nectans</i>	<i>E. coli</i>	<i>E. freundii</i>	<i>Proteus mirabilis</i>	<i>Alkaligenes faecalis</i>	<i>C. histolyticum</i>	<i>C. paraputrificans</i>	<i>C. histolyticum and C. paraputrificans</i>
Female	13	11	1	—	1	—	—	—	5	3	2
Male	33	20	3	1	3	2	1	3	7	4	11
Total	46	31	4	1	4	2	1	3	12	7	13

- 5) 1.0 ml of *Cl. histolyticum* broth culture + 1.0 ml *Staphylococcus* broth culture + 1.0 of *Escherichia coli* broth culture.
- 6) 2.0 ml of *Cl. lentoputrescens* broth culture.
- 7) Controls were injected with dose amount of the medium.

Stool specimens were collected in sterilized containers and suitable media, mentioned elsewhere, were inoculated for the isolation of aerobic and anaerobic bacteria and incubated accordingly at 37°C. Biochemical and fermentation reactions in conjunction with serological differentiation procedures were performed for the identification of isolates (Bergey's Manual, 1957). Toxigenicity of the isolates was also determined.

Results and Discussion

Stool samples were collected from 46 random patients out of over 200 studied cases belonging to different age and sex groups with symptoms of acute diarrhea, during and after syndrome of disease. The distribution of organisms, with respect to age is recorded in

Table III: Toxigenicity Tests

No.	Cultures	No.	Control Rabbits Remark	No.	Test Rabbits Remark
1	<i>C. histolyticum</i>	2	All rabbits remained healthy after 4 weeks	12	Necrosis of tissues within 15 hours (area of digested muscles was $1\frac{1}{2} \times \frac{1}{2}$)
2	<i>Vibrio cholerae</i>	2	"	29	All rabbits died within 12 hours
3	<i>Cl. histolyticum</i> + <i>Vibrio cholerae</i>	2	"	4	All rabbits died after 15 hours
4	<i>Cl. histolyticum</i> + <i>Proteus inconstans</i>	2	"	4	All rabbits remained healthy after 4 weeks
5	<i>Cl. histolyticum</i> + <i>Staphylococcus</i> + <i>E. coli</i>	2	"	4	"
6	<i>Cl. lentoputrescens</i>	2	"	4	"

Table IV: Micro-Organisms Found in Stool of Patients After Recovery of Diarrheal Syndrome

Age (in years)	No. of Patients	<i>Proteus Mirabilis</i>	<i>Proteus inconstans</i>	<i>Escherichia coli</i>	<i>Staphylococcus aureus</i>	<i>Staphylococcus albus</i>	<i>Staphylococcus citreus</i>
1-10	14	2	—	5	—	1	5
11-20	8	—	—	5	—	—	1
21-30	9	1	1	3	—	—	3
31-40	6	—	—	1	2	—	3
41-50	5	1	—	1	—	1	2
51-60	2	—	—	1	—	1	—
61-70	1	—	—	—	—	—	—
71-80	1	—	—	—	—	—	—

Table I and with respect to sex is recorded in Table II. Results of toxigenicity tests are recorded in Table III. Micro-organisms found

in stool sample collected after diarrhea syndrome are summarized in Table IV.

Vibrio cholerae was incriminated as main causative agent of diarrhea studied and isolated from 31 cases (67.39%). This agent had been responsible for many outbreaks in many Asiatic countries (Cruickshank 1968; Dubos 1958). Few cases of other vibrios were also reported *Vibrio percolans*, inhabitant of water, was isolated from 4 cases (8.70%). *Vibrio neocistes*, inhabitant of soil, was isolated from one case (2.17%). Their association with the disease has not been reported so far and they were considered non-pathogenic (Bergey's Manual, 1957). *E. coli* was associated with infantile diarrhea and traveller's diarrhea (Bennington et al., 1972; Gorbach et al., 1975). In the present study, 4 cases (8.7%) were associated with *E. coli* and in 2 cases the causative was *E. Freundii*, which was suspected to cause enteric infection, though its etiological relationship could not be established (Dubos 1958; Cruickshank 1968). *Proteus inconstans* and *Alkaligenes faecalis* had been reported to be related to gastroenteritis (Bergey's Manual, 1957). In the present study, 1 (2.17%) and

3 (6.52%) cases were isolated with these agents respectively. Anaerobic studies of the stool cultures revealed 2 types of organisms; both

belonged to the genus *Clostridium*. *Clostridium histolyticum* was isolated from 12 cases (26.01%), *Cl. lentoputrescens* from 7 cases (15.22%), 13 (28.26%) cases were positive for both the organisms. *Cl. lentoputrescens* was found to be a normal inhabitant of intestine while *Cl. histolyticum* was found occasionally in faeces (Bergey's Manual, 1957; Bennington et al., 1972).

Infectious diarrhea was thought to be a disease of children (Bennington et al., 1972). In Arizona 67% cases belonged to persons less than one year old and bacterial pathogens were isolated by a single examination from 57% cases (Goodwin et al., 1960). In present study 30.4% cases were isolated from children. It was interesting that persons with ages more than 50 years were less susceptible to the infection. Hence only 8.69% cases could be isolated from that age groups. Similarly male (71.74% positive cases) were found susceptible to the infection. Only 28.26% female fell victim to the disease.

No species belonged to *Solmonella* and *Shigella* group of organisms. The studies for the isolation of enterovirus could not be conducted. However, their role could not be neglected and further investigation on this aspect is suggested.

Cl. histolyticum, isolated in the study, produced a potent exotoxin which possessed a necrotic activity for the tissues (Table III). Exotoxin produced by *V. cholerae* had lethal effect for rabbits. It is striking that the activity of the toxin produced by *Cl. histolyticum* was inhibited in the presence of other enteric organisms. This phenomenon might be an explanation for normal activity of these *clostridia* present in intestine under normal conditions, though isolation of proteolytic *clostridia* from faeces had been reported (Dubos 1958).

A study for the isolation of prevalence of micro-organisms after recovery of such diarrheal syndrome was also carried out and stool cultures of infected persons were repeated after syndrome. *P. mirabilis*, *P. inconstans*, *E. coli*, species of *Staphylococcus* group were isolated (Table IV). These organisms had been reported previously as normal inhabitant of intestine of healthy persons (Cregan & Hayward, 1953; Kalser et al., 1966; Bennington et al., 1972).

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