MATERIAL AND METHODS
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This study was carried out in the department of Paediatrics and Microbiology, N.I.E. Medical College Hospital, Jhansi. A total of 109 children in the age group of 1 to 60 months with acute diarrhoea were studied. Cases were selected from Paediatrics out-patient clinic and Paediatric ward.

One hundred age and sex matched non-diarrhoeal controls were selected from admitted patients for minor illnesses like Asthmatic bronchitis, epilepsy, etc. and from asymptomatic siblings of admitted patients, with no history of diarrhoea in last 15 days.

A detailed history was recorded and clinical assessment and microbiological investigations were carried out as detailed below.

HISTORY

A detailed history was taken from mother or a family member of the patient in particular for duration of illness, quantity, frequency, and consistency of stools, Duration and frequency of vomiting, presence of fever, pain in abdomen, tenesmus, duration of period when last urine is passed, for any complication like seizure, abdominal distension, anuria etc. A detailed history was recorded from mother regarding pattern of feeding. Whether baby is bottle fed, breast fed or mixed fed (bottle + breast), or fed by cup and spoon. Attempt was
also made to judge the standard of bottle hygiene by questioning the mother, and details of medication and fluid therapy taken earlier was also recorded.

**PHYSICAL EXAMINATION**

A detailed physical examination was carried out. This included a preliminary general examination. The degree of dehydration was assessed according to well known criteria of WHO. Apart from this, the child was also examined for presence of fever, URI, oral thrush, perianal excration or depigmentation for any septic focus. The routine systemic examination was carried out.

Assessment of nutritional status was done according to classification of Indian Academy of Paediatrics.

**COLLECTION OF SAMPLE**

Rubber capped clean glass vials were supplied to the mothers so that they could collect a part of stool sample of their children in the morning just after defaecation. The vial with specimen was labelled and was brought to the laboratory within 2 hours.

**MACROSCOPIC EXAMINATION**

The sample was examined for the consistency of the stool, presence of blood, mucous and adult parasites by naked eye.
MICROSCOPIC EXAMINATION

Direct microscopy was done in normal saline preparation and Lugol's iodine preparation. On a clean glass slide 2 drops of normal saline on side and 2 drops of Lugol's iodine on the other side were placed. With a match stick a small portion of faeces (about 2 mg) was picked up and emulsified in each of the drops. The smears were covered with cover slips and examined for ova, trophozoites and cysts under low (100 x) and high (400 x) magnification. Erythrocytes and pus cells were also looked for.

CULTURE

All the specimens were plated on Mac Conkey agar (MA), deoxycholate citrate agar (DCA) and thiosulphate citrate bile salt sucrose (TCBS) agar plates. The plates were incubated at 37°C over night. MA and DCA plates were examined for lactose fermenting (LF) and lactose non-fermenting (NLF) colonies. LF colonies of pure and predominant type (more than 80% colonies looked alike) and all NLF colonies were processed. TCBS agar plates were looked for flat yellow colonies of vibrio cholerae and green mucoid colonies of V. parahaemolyticus. One suspected colony was picked up and inoculated in 2 ml peptone water. After 3-4 hours of incubation in peptone water each culture was inoculated with a straight wire into triple sugar iron agar (TSI), sulphide indole motility medium (SIM), Christensen's urea, Simmon's citrate, Falkow's decarboxylase broths and
peptone water sugars, like glucose, sucrose, mannitol and lactose. Oxidase and catalase tests were also performed. All the strains were identified following Cowan and Steel (1974). The strains were confirmed by agglutination test with specific antisera.

For isolation of Campylobacter, Blaser's medium was used. After inoculation, the plates were incubated at 42°C in a candle jar for 48 hours. After incubation, the plates were looked for either small discreet greyish colonies or irregular, watery colonies running along the wire track. The suspected colonies were confirmed by Gram's stain, motility, catalase, oxidase hippurate hydrolysis and no growth at 25°C and in 3.5% NaCl.

DETECTION OF ROTAVIRUS

On receipt of sample in the laboratory the procedure of preparation of faecal extract and detection of rotavirus antigens in them by the ELISA technique was according to the manual provided with the kit of reagents (The ELISA kit was from WHO collaborating centre on Human Rotaviruses, Birmingham Hospital, Birmingham, U.K.).

About 1 gram of faeces was taken in a centrifuge tube and emulsified in 10 ml of sterile phosphate buffered saline (PBS) at pH 7.3. The emulsion was centrifuged by low speed centrifugation i.e. approximately 2000-3000 g for 10-15 min. After centrifugation the supernatant (which is 1/10 dilution of faecal extract) is collected in
screw capped bottle and stored at -20°C till sufficient number of samples had accumulated.

The procedure of the screening ELISA test is briefly as follows. A flat bottomed micro-titre plate (NUNC) was coated with 0.1 ml of 1 : 10000 of rabbit antirotavirus serum at 4°C overnight. Then the plate was washed six times with phosphate buffer saline with Tween 20 (PBS/T). The test sample was then added (0.025 ml + 0.075 ml of buffer) to duplicate wells and incubated at 4°C overnight. Known positive (near and 1/10 diluted) and negative controls were included in addition to substrate and conjugate controls. The plate was then washed six times in PBS/T as before. Then 0.1 ml of 1 : 10000 guinea pig anti-rotavirus serum was added to each well except substrate and conjugate controls. The plate was sealed and incubated at 37°C for 2½ hours. The plate was washed again and 0.1 ml of 1 : 800 goat anti-guinea pig antibody labelled with alkaline phosphatase (conjugate) was added. After incubation at 37°C for 1½ hour and washing 0.1 ml of substrate containing 1 mg/ml of p-nitrophenyl phosphate was added to each well. After 10-20 minutes, when the diluted positive control wells had developed a yellow colour, the reaction was arrested by addition of 0.05 ml of 3N sodium hydroxide. The results were read by naked eyes.