Role of Toxigenic Bacteria in Acute Infantile Diarrhoea

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Abstract

The cause of infantile diarrhoea was investigated with the use of Biken test and animal assay for toxigenic bacteria. EnterOtoxigenic Escherichia coli (ETEC) was recovered in 39 cases (17.3%). Of these 30 patients (13.3%) were infected with heat-labile (LT+ETEC) and 7 (3.1%) with heat-stable producing ETEC (ST+ETEC) and 2 (0.9%) with both toxins producing strains (LT+/ST~ETEC). Majority of cases in this series were males between 2-3 years of age (JPMA 34,266 :1984).

Introduction

Enterotoxigenic E.coli are responsible for acute diarrhoea in infants, children, adults and travellers[1-3] ETEC produces a heat-labile (LI), heat-stable (SI) or both.toxins. LI enterotoxin is immunologically similar to cholera toxin[4] while SI enterotoxin is non immunogenic[5].
ETEC has been identified as an important cause of infantile and young childhood diarrhea in many developing[1,6,7]. In these studies the number of.cases examined was small due to limitation and complexity of the assay system to determine the enterotoxin pro duction. However, the recently developed simple and reliable, BIKEN test can solve the problem, as seen in the present study.

Material and Methods

Faecal specimens were collected from 225 patients under three years of age admitted to Central Government Polyclinic Hospital Islamabad during summer season of 1982. All patients had sudden onset of watery diarrhoea within 24 hours prior to admission with moderate to severe dehydration. Bacteriological examination was negative for Salmonella, Shigeila, and vibrios.
Specimen was plated on MacConkey agar (Difco). After overnight incubation four lactose fermenting colonies were randomly picked and identified[9] and then tested for enterotoxin production.
Assay'For E. Colt Entero toxin Heat-labile toxin was detected by BIKEN test[8]. This test is carried out on a special medium (Biken agar No. 2.).

Preparation of Biken Agar No. 2.

1. Mix 2% casamino acid, 1% yeast extract, 0.25% Naci and 1.5% K_2HPO_4, 0.5% Glucose, 0.05%, trace salt solution (5% $Mgso_4$' 2% Cad_2 ' $6H_2O$ and 0.5% Fecl)
2. Adjust pH to 7.5 withLM NaoH.
3. Add 1.5% Noble agar and boil until it melts.
4. Autoclave at 15Lb, 121 °C for 15 minutes.
5. Cool to 50 °C and add lincocin to final concentration of 90 ug/ml and mix well.
6. Pour 15 ml of medium to each plastic petridish (90x15mm).

Test

On each petridish of Biken agar No.2 E. coli isolates were inoculated to ensure a fairly large area of confluent growth (Fig. 1)

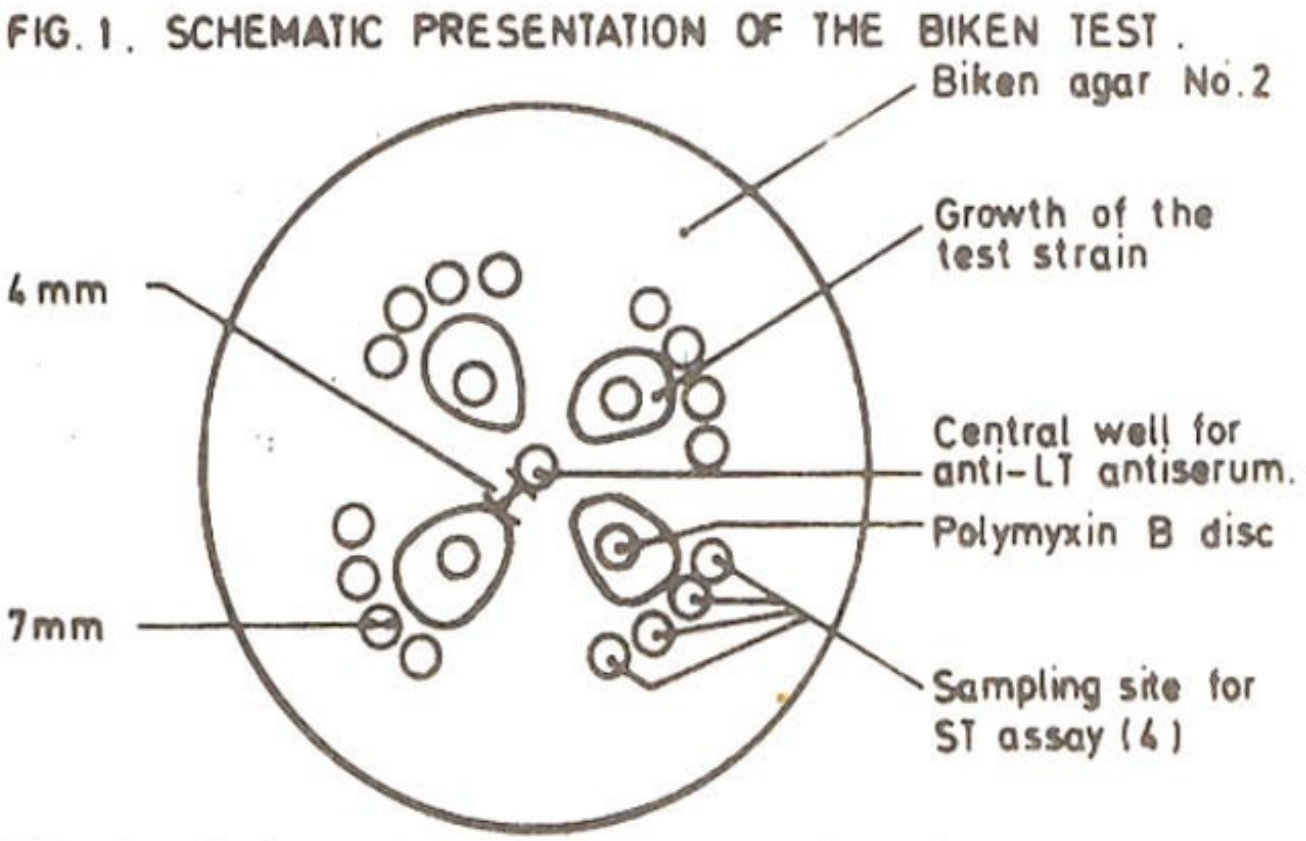

Fig. 1 Schematic presentation of the Biken test.

around the site where central well will be punched.
After 48 hours of incubation at 37°C a polymyxin B disc containing 20,000 IU/ml was placed, on the top of the growth of each strain.
A well was punched in the centre of the area so that the distance between the well and the growth was about 4mm and Incubated again for 5 -6 hours. 20 ul of the optimal dilution of the antiserum against LT was placed into the central well and Incubated again for 20-24 hours.
Precipitation line was examined in the zone between the growth and the central well (Fig. 2)

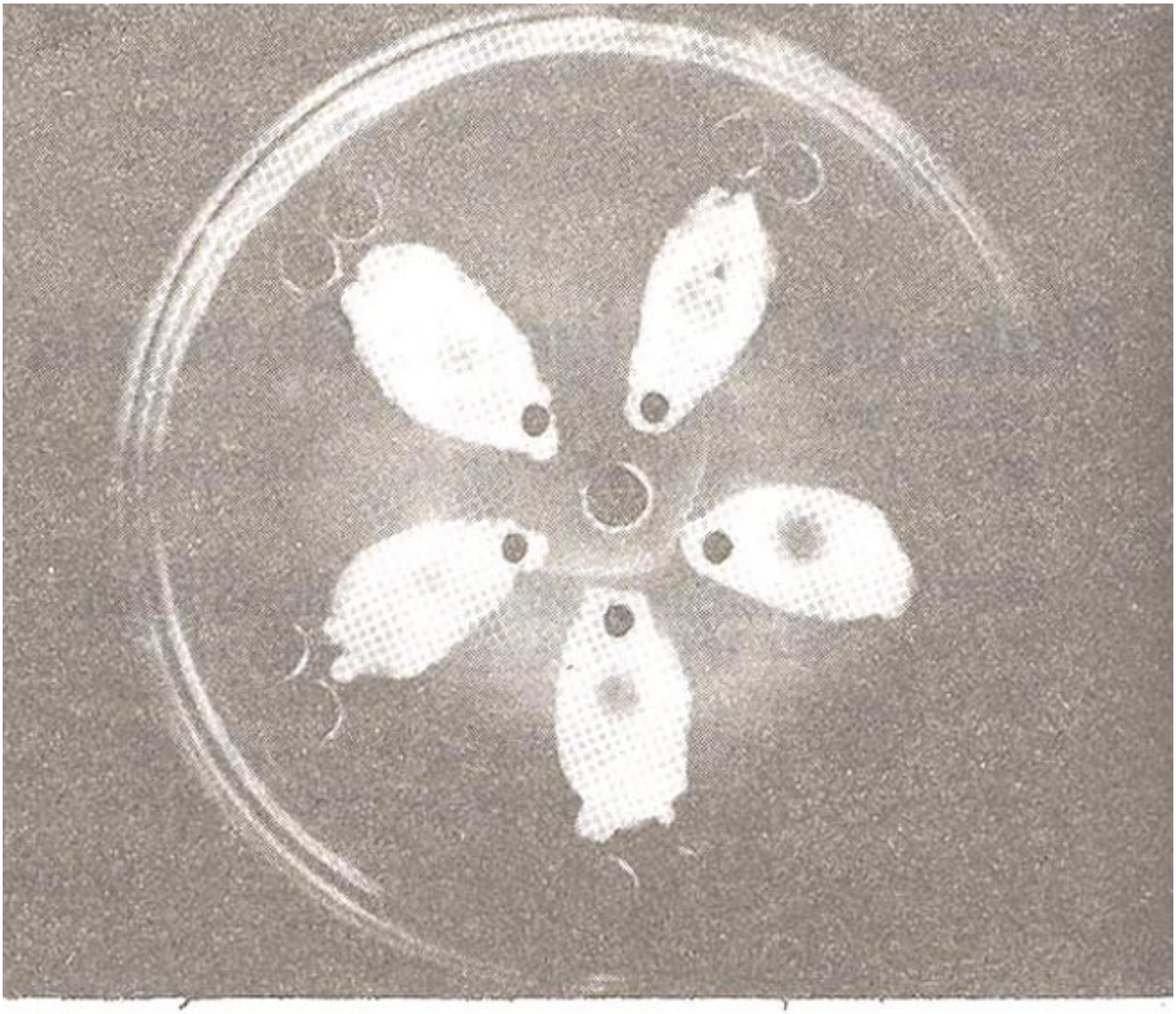

Fig.2 Two strains of enterotoxigenic E.coli showing precipitation line around the central well.

by placing the plates on a light box with black background.
Positive E. coli strain No. 240-3 and negative strain No. 212-5 were used as control with each batch of the test.

Sampling for ST

After Polymyxin B treatment, and just before the anti-LT antiserum was placed in the central well, 4 pieces (7mm in diameter) were punched out from just outside the periphery of each colony (Fig. 1). These pieces were placed into 0.5 ml phosphate buffered saline (0.01 M pH 7.0) and left overnight at 4°C to extract the ST. 0.1 ml of this fluid per suckling mouse was used for ST detection.

Suckling Mouse Assay for Heat stable (ST) Toxin:

One drop of 2% Evans blue was added to the fluid prior to suckling mice assay. One ml tuberculin syringe (fitted with a small teflon tube at the needle tip) was filled with this fluid. 1-3 day old suckling mice were separated from their mothers immediately prior to use and randomly divided into groups of three. Each mouse was given 0.1 ml of this fluid[10]. Inoculated mice were placed at room temperature

for four hours, then killed by using chloroform. The abdomen was opened and the entire intestine (Distal to stomach) was removed with forceps. The intestine was weighed and the ratio of intestine to total remaining body weight of 0.083 were considered positive (Fig. 3).

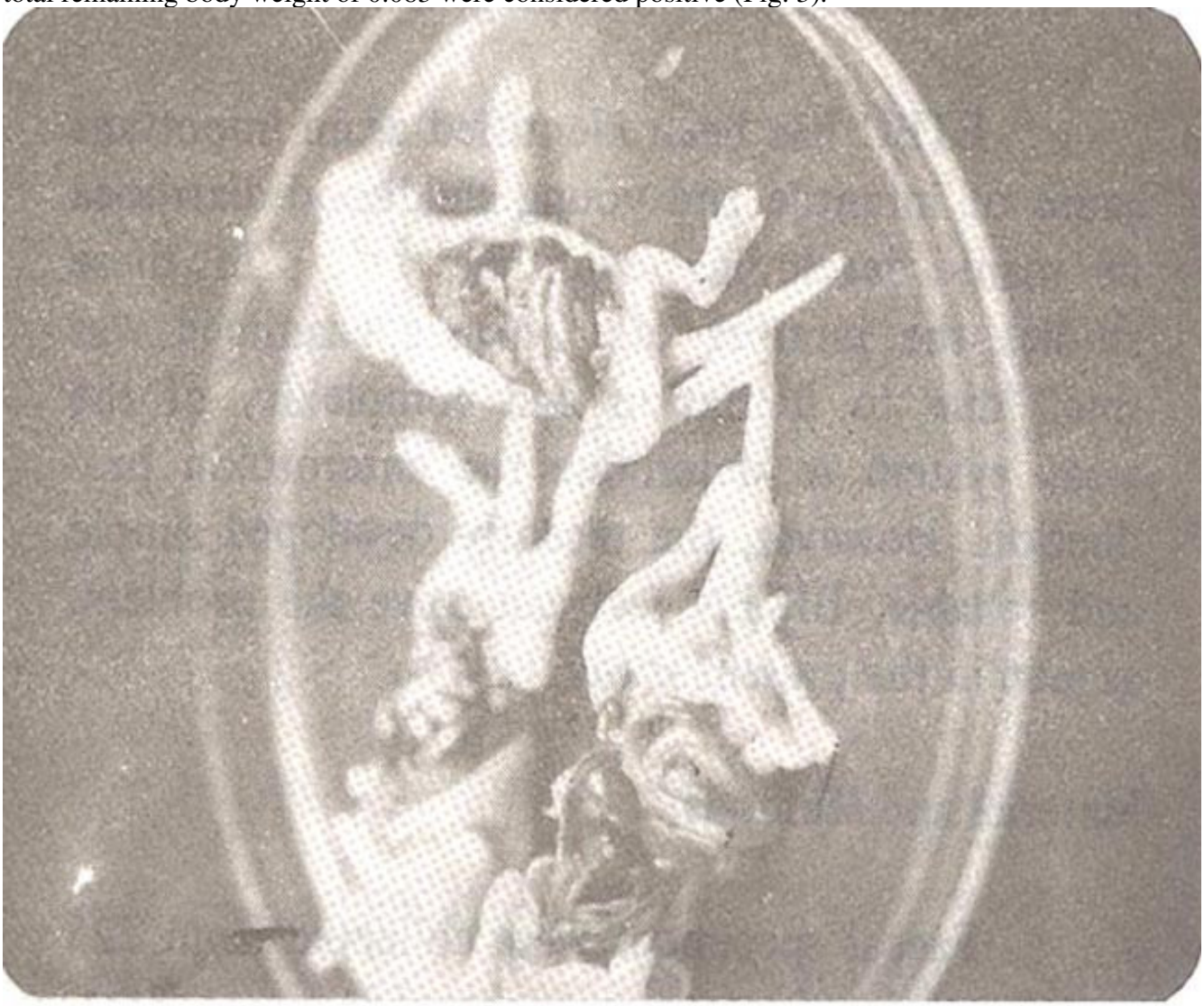

Fig. 3 Fluid accumulation in intestine of two suckling mice indication, the positive test for heat-stable toxin of E. coli.

Animals with no dye in the intestine or aye within peritoneal cavity at autopsy were discarded.

Result

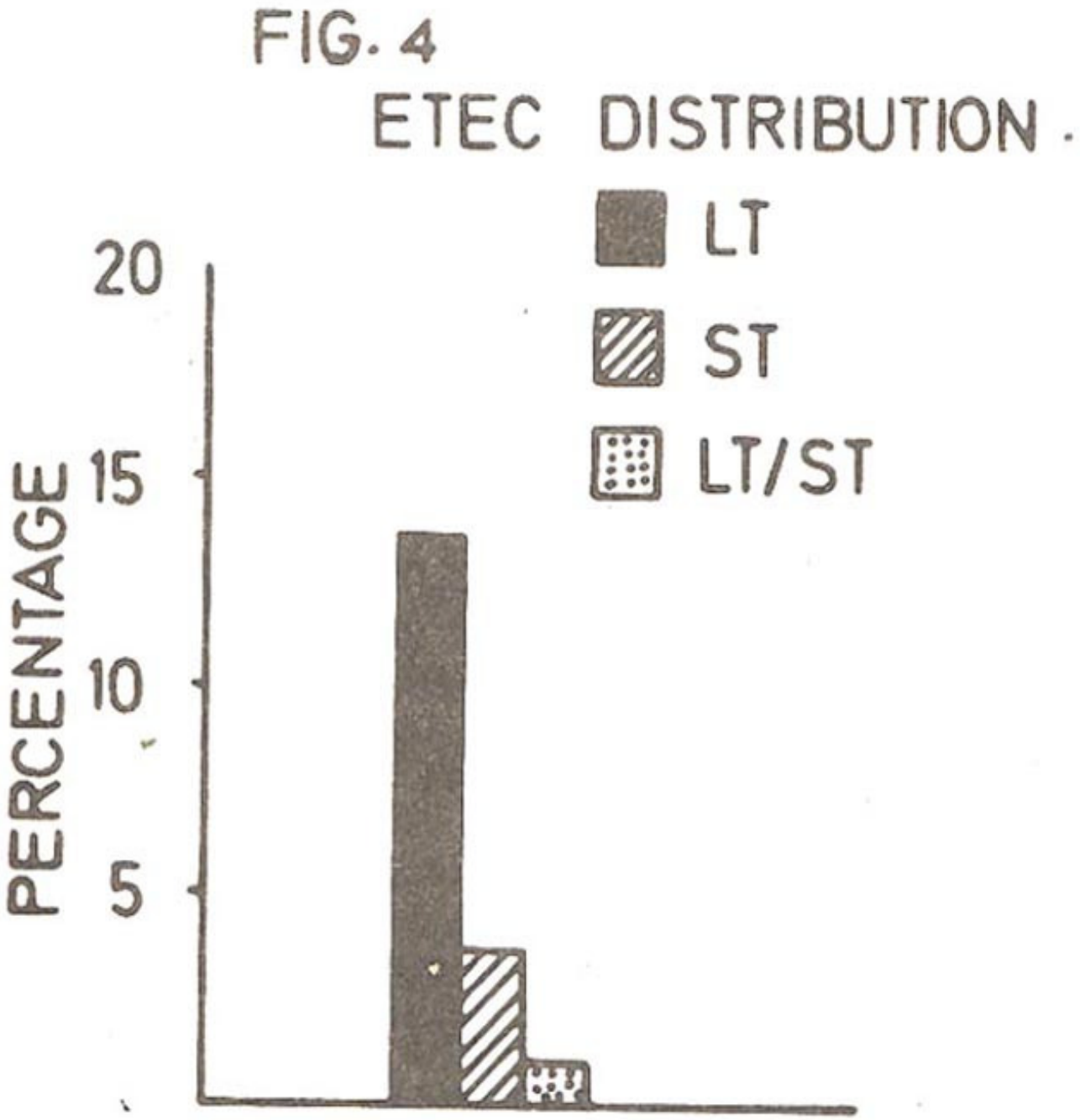

Figure 4 shows the frequency of enterotoxigenic Escherichia coli as an aetiological agent of infantile diarrhoea in 225 faecal specimens. Diarrhoea due to ETEC comprising 17.3% of total cases. Among ETEC heat-laoile toxin producing strains were more frequent (13.1%). Only (0.9%) cases were infected with both toxins (LT and ST producing ETEC).

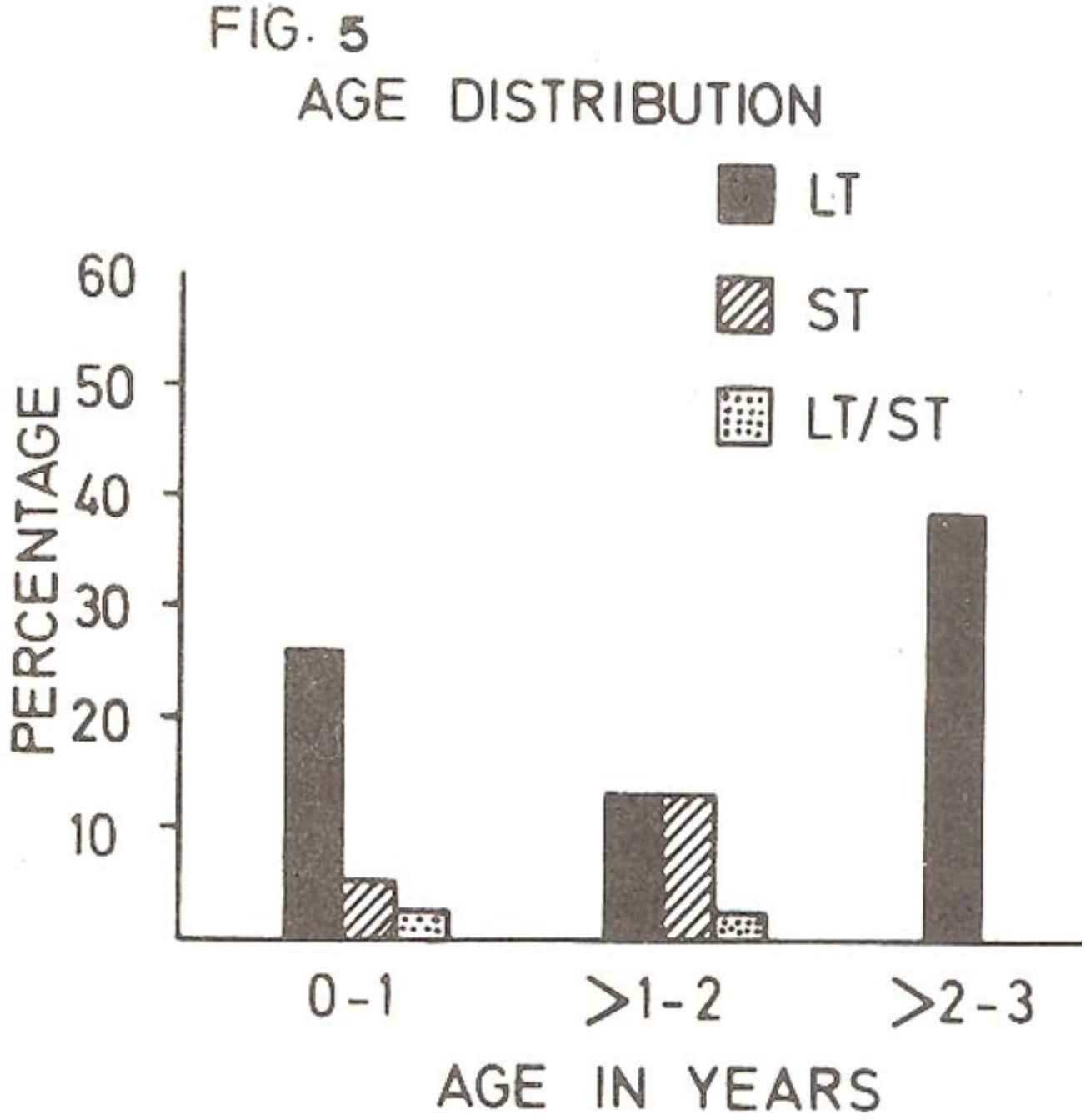

Fig. 5 Age distribution.

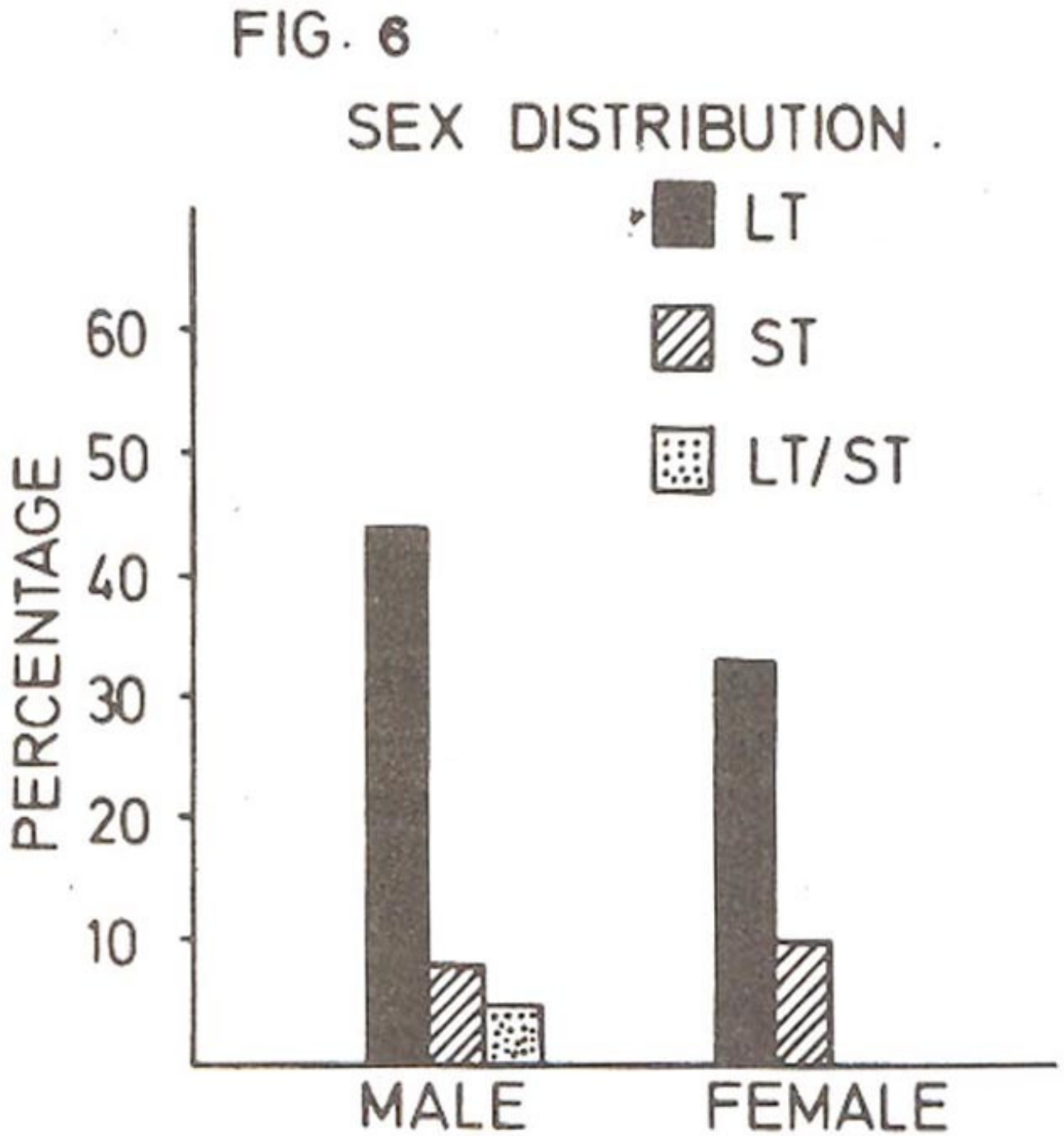

Fig.6 Sex distribution.

Figure 5 and 6 show the age and sex of the patients respectively. Diarrhoea due to LT was slightly more frequent in age group of 2-3 years. However, no significant difference was found between age group and isolation of ETEC. Generally ETEC diarrhoea was more common in males. However, ST producing ETEC mainly affected the females while LT+/ST+ strains were responsible for diarrhoea only in males.

Discussion

The .requency and potential severity of diarrhoea in infants and children throughout the world is well known.[11] Within the last decade ETEC have been tound to be an important cause of acute diarrhoea in developing countries. However, the exact nature and extent of the problem could not be determined due to lack of adequate facilities for laboratory diagnosis of enterotoxigenic E. coli.

Recent development of Biken test made it possible for a small laboratory to recognise ETEC as an aetiological agent of diarrhoea.

Previous studies have shown that the most frequent aetiologic factors associated with diarrhoeal illness in children were entero-pathogenic E. coli (EPEC)[12] and Shigella[13]. While in this study main emphasis was given on the detection of ETEC which provides an evidence that such organism may be responsible for quite a proportion of acute diarrhoea in this population.

ETEC infection has varied regional valence in diarrhoeal patients. Eighty percent of hospitalized children with diarrhoea in Chicago ana only 18% Apache children15 were infected with ETEC. In this study 17.3% children had diarrhoea due to ETEC infection.

Frequency of diarrhoea due to LT producing ETEC was lower (7%) in Taiwan and Philippines[16,17] and slightly higher (13.3%) in this series. ST associated diarrhoea was responsible only for 3.1% of cases, which is in contrast to other[7,18] studies which report a higher frequency

ETEC diarrhoea affects all age groups but more frequently 10 year old children with the predominance of ST producing ETEC[19]. In the current series LT associated diarrhoea was mainly found in age group of 2-3 years, where as, it is mostly reported in patients below one year of age[20].

In most of the series from developing countries, the number of cases examined for ETEC has been small and data available suggest possible geographic differences in the relative frequency of LT/ST, LT, ST producing strains.[1,20,21] The reason for differences in the distribution of toxin types is not clear.

In present study ETEC has emerged as a potential aetiological agent of infantile diarrhoea in this region, however, the study in all age group will provide the actual prevalence of ETEC in the population.

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