elderly woman had left colon diverticula and right colon angiodysplasia but which was the culprit could not be established. A blind subtotal colectomy which could take care of both lesions was deemed unwise in her frail physical state. Angiodysplasia is still very rare among blacks.

In summary, our study confirms the previous report from Johannesburg that diverticular disease is emerging among urban blacks in South Africa and further draws attention to haemorrhage as a predominant presenting feature. These findings might equally apply to other newly urbanised communities who have historically been free of this disease. The increasing global mobility of patients and clinicians requires the latter to be familiar with different patterns of disease and their presentation in different races and communities; this will help prevent mortality caused by missed diagnoses.

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Incidence of heat-labile enterotoxin-producing *Escherichia coli* detected by means of polymerase chain reaction amplification

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Abstract **Diarrhoea can be caused by many different organisms, some of which are notoriously difficult to identify. One of these is enterotoxin-producing *Escherichia coli*. Recently a new diagnostic technique that uses polymerase chain reaction DNA amplification was developed for detection of the 'A' subunit of the labile enterotoxin-producing *E. coli* gene. This technique was used to evaluate the incidence of heat-labile (LT^+) enterotoxin-producing *E. coli* in the causation of diarrhoea.**

The results from this study showed that LT^+ *E. coli* is a cause of diarrhoea in the western Cape and that 5,3% of non-diagnosed diarrhoea patients in Tygerberg Hospital were infected with this pathogen. This represented less than 1% of the total number of cases of diarrhoea investigated in this hospital. The peak coincides with the wetter months in this locality and the infection rate is lower than that reported in most other countries. Given the low incidence of occurrence of this organism we do not recommend routine implementation of the diagnostic procedure. However, this test may be useful at times, e.g. to ascertain the source of a diarrhoea epidemic.

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Acute diarrhoea has a significant impact on public health[1] (particularly among children in developing countries). Previous studies have shown that rotavirus and *Salmonella* spp. are among the common causes of diarrhoea in urban areas, whereas enterotoxigenic *Escherichia coli*, *Shigella* spp. and other bacterial agents are a more important cause of diarrhoea in rural areas, where the water supply and public health measures are often poor.[2] *E. coli* is reported to be one of the most important causes of diarrhoeal illness among children and adults in developing countries and in the industrialised world.[3]

Numerous techniques are available for the identification of these aetiological organisms — including *E. coli*. Most often, MacConkey and blood agar plates together with serological typing are used for the diagnosis of *E. coli*.[4] Techniques for the direct identification of enterotoxigenic *E. coli* or gene products include the following: (*i*) tissue culture and skin permeability factor assay;[5] (*ii*) Y1-adrenal cells in tissue minicultures;[6] (*iii*) direct serological assay with passive immune haemolysis;[7] and (*iv*) identification by means of enzyme-linked immunosorbent assay with antisera against labile toxin after bonding to specific antibodies or gangliosides.[4] Techniques aimed at the detection of specific genes such as DNA colony hybridisation,[8] direct detection of enterotoxin-producing genes with radio-active DNA probes[9] and non-radioactive polynucleotide gene probe assay for identification of enterotoxigenic *E. coli*,[10] were also used for identification.

However, serological typing and tissue culture techniques have been found to be labour-intensive as well as nonspecific and insensitive.[10] The use of radiolabelled

cloned DNA probes and oligonucleotides in colony hybridisation for heat-labile (LT^+) and stable (ST^+) toxin genes is not very sensitive. These methods are fairly sophisticated and, although specific,[8,10-12] are not suitable for routine laboratory diagnosis. More recently a new technique, polymerase chain reaction (PCR) amplification of DNA, was developed for the amplification of the 'A' subunit of the LT^+ toxin gene, without prior DNA extraction.[11]

Detection of the amplified product is possible with polyacrylamide gel electrophoresis, which is sensitive enough to detect the LT^+ enterotoxin gene in stool-derived samples. The PCR technique described is relatively sensitive, specific, simple and inexpensive.

Since diarrhoea is such an important disease (particularly among children), this study was undertaken to evaluate the role of LT^+ enterotoxin-producing *E. coli* in the causation of diarrhoea in patients at Tygerberg Hospital.

Materials and methods

Stool specimens

For the amplification of the LT^+ toxin gene, stool specimens from patients with severe diarrhoea were obtained. These were collected over a period of 12 months (January - December 1990). The specimens used for this study were selected according to the following criteria: (*i*) the stool specimens were all from patients with severe diarrhoea; (*ii*) no *Shigella* spp., *Salmonella* spp., *Campylobacter* spp., *Klebsiella* spp., *Staphylococcus* spp. or any parasites were identified during routine screening for the abovementioned organisms by the microbiological laboratory at Tygerberg Hospital; and (*iii*) there had to be overgrowth of *E. coli* after overnight incubation on MacConkey plates. After the MacConkey plates had been selected, they were directly tested for the presence of LT^+ enterotoxin-producing *E. coli* by means of the PCR amplification technique described below.

Patient data

Information was obtained from the hospital files of those patients whose stool specimens proved to be LT^+ *E. coli*-positive after the PCR analysis.

Detection of LT^+ toxin-producing *E. coli* by means of PCR amplification

PCR amplification with gene-specific primers was essentially done as described in earlier studies.[11] A scraping of *E. coli* from the MacConkey plates was diluted in 400 µl of sterile saline and boiled for 20 minutes. Ten microlitres of this lysed and denatured suspension of bacteria were combined in a total volume of 99 µl with a premixture of 1 × PCR buffer (10 mM Tris-HCL [pH 9,0 at 25°C], 1,5 mM $MgCl_2$, 50 mM KCl, 0,01% gelatin (w/v), 0,01% Triton X-100), a 200 µM final concentration (each) of dATP, dCTP, dGTP and dTTP and 0,4 µM of each primer. After the addition of 0,5 U of Taq DNA polymerase (Promega), the mixture was overlaid with 40 µl of mineral oil and the heating cycle of 93°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes was repeated 35 times in a thermal cycler. The amplified product was analysed by means of electrophoresis on ethidium bromide containing 12% polyacrylamide gels, and the DNA was visualised by means of transillumination. LT^+ enterotoxin-producing *E. coli* (ATCC 43886) and sterile saline were used as positive and negative controls respectively.

Results

Detection of LT^+ *E. coli* by means of PCR analysis

Clinical isolates were analysed over a period of 1 year by means of culture, PCR and the electrophoresis system. Fig. 1 is a sample result and indicates that LT^+ *E. coli* could be a cause of diarrhoea in the western Cape. Some negative samples were spiked with a few LT^+ bacteria and analysed again. Positive results were obtained thereafter, which indicated that a negative result was not due to the presence of polymerase inhibitors in the samples tested. A negative result was obtained in each batch of samples for the control tube containing saline instead of template (Fig. 1, lane 10). Seventeen out of 323 samples were positive for LT^+ enterotoxin plasmid (5,3%) (Table I).

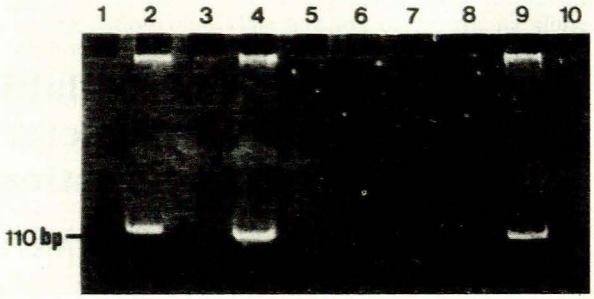

FIG. 1.
Detection of LT^+ enterotoxin-positive *E. coli* in the clinical isolates. The stool samples where no enteropathogen could be identified after routine screening were inoculated on MacConkey plates. A scraping from this colony growth was directly amplified with primers LT31 and LT51. Twenty-microlitre samples of the amplified product were used for gel electrophoresis and detection with ethidium bromide. A 12% polyacrylamide gel was used. The sample in lane 10 contains no template DNA, only sterile saline. The numbers on the left indicate molecular sizes in base pairs. Lanes 2, 4 and 9 are the only lanes which contain LT^+-positive samples.

Patient data

Patient data are presented in Table I. All the patients, although admitted for different reasons, had severe diarrhoea. Most of these patients were under 5 years old. Thirteen of the admissions were during the months March to June. More female patients (13) had diarrhoea than male patients (4); most of these patients were coloured ($N = 14$). Only 3 black and no white patients were LT^+ *E. coli*-positive. On admission most of the patients were diagnosed as having gastro-enteritis, malnutrition (including marasmus and kwashiorkor) and necrotising enterocolitis. The patients spent between 2 days and 28 days (average 11 days) in hospital before either being discharged or before the diarrhoea disappeared. These patients were not treated for *E. coli* infection specifically, since the results obtained were generally not available to the clinicians treating the patients at the time of hospitalisation.

TABLE I.
Incidence of LT⁺ enterotoxin-producing *E. coli* over a period of 1 year

Month of admission	PCR-positive cases	Race	Sex	Age (yrs)	Diagnosis made on admission
Jan	0				
Feb	0				
Mar		C	M	4	Gastro-enteritis
		C	F	2	Gastro-enteritis
	5	C	F	1	Gastro-enteritis, malnutrition, anaemia
		C	M	12	Lymphoma
		C	F	< 1	Necrotising entero-olitis, septicaemia
Apr	2	B	F	1	Gastro-enteritis, malnutrition,
		C	F	2	Gastro-enteritis
May		B	F	1	Gastro-enteritis, malnutrition,
	4	B	F	1	Gastro-enteritis
		C	F	1	Gastro-enteritis, malnutrition
		C	F	52	Diverticulitis
Jun	2	C	F	1	Gastro-enteritis
		C	M	1	Gastro-enteritis
Jul	1	C	F	24	Chronic diarrhoea
Aug	1	C	F	< 1	Jaundice
Sep	1	C	F	1	Gastro-enteritis
Oct	1	C	M	< 1	Gastro-enteritis
Nov	0				
Dec	0				

A total of 323 selected samples was analysed, of which 5,3% tested positive for LT⁺ enterotoxin-producing *E. coli*. The race group classification refers to the so-called coloured (C) and black (B) races respectively.

Discussion

Some diagnostic laboratories have reported that there are many cases of diarrhoea (e.g. 40%) where the pathogen could not be identified.[13] The aim of this study was to determine the incidence of LT⁺ *E. coli* and the role it played in the causation of diarrhoea at Tygerberg Hospital where the pathogenic organism was not diagnosed.

The results presented in this study show that 5,3% of non-diagnosed diarrhoea patients were infected with this pathogen. This represents less than 1% of all cases (various causes) of diarrhoea investigated in this hospital. The predominance of female patients is in contrast to the male dominance found in Ibadan, Nigeria.[14] The incidence is higher than the 2,6% LT⁺ *E. coli* found in Seoul, South Korea,[1] and the 2,5% *E. coli* found on the island of Hong Kong,[2] but is lower than the 16,6% LT⁺ *E. coli* found at Bandar-Abbas, Iran,[15] the 24,3% previously reported for both LT⁺ and ST⁺ toxins in a different region in South Africa[16] and the 45,3% LT⁺ *E. coli* found at Lugar Sobre la Tierra Blanca, Mexico.[17] The reason for this difference could be the differences in the sampling of the study population or the differences in the methods used to identify the LT⁺ *E. coli*. Pathogenic *E. coli* is found mainly where water supply and public health services are poor and in densely populated areas. Variation in health serving and urban behaviour could also explain differences in infection rates.

The high incidence of admissions during the months of March to June coincides with the wetter autumn and winter months in this locality. An annual seasonal peak of infection with this enteropathogen in Mexico also occurs in April to July;[18] however, in Seoul, South Korea, the seasonal peak is during the dry months of October and November.[1]

The low incidence of this organism at Tygerberg Hospital does not justify the expense of routinely assaying all diarrhoea patients; this method may nevertheless be useful at times, e.g. to determine the cause of a diarrhoea epidemic.

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