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Detection of pathogenic bacteria in children with diarrhoea

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86 children ranging from one to three years old with diarrhoea from Yangon Children's Hospital and respective controls were included in this study. Isolation rates of major pathogens responsible for acute diarrhoea in children were Enterotoxigenic *Escherichia coli* (ETEC) 30.2 per cent, Enteropathogenic *E. coli* (EPEC) 8.1 per cent, *Salmonella* 1.2 per cent, *Shigella sonnei* 1.2 per cent, *Plesiomonas* 1.2 per cent and vibrios 1.2 per cent. In the control group the prevalence was found to be ETEC 9.3 per cent, EPEC 7.0 per cent, *Salmonella* 12.0 per cent, *S. Sonnei* 1.2 per cent, *Plesiomonas* 7.0 per cent and vibrios 1.2 per cent. The total pathogen isolated from diarrhoea and control cases were 46.5 per cent and 38.4 per cent respectively. It was also noted that *Salmonella* and *Plesiomonas* isolation rates were higher in the control group.

INTRODUCTION

During the Multicentre Hospital based control study on the aetiological agents responsible for acute diarrhoea in children during the years 1982 and 1983, it was found that most of the bacterial agents were Enterotoxigenic *E. coli* out of the 7 major pathogens tested. There was a remarkable decrease in the isolation rate of other pathogenic organisms besides Enterotoxigenic *E. coli*. Thus, we are again aiming to find out a new pathogen besides 7 major pathogens which might be responsible for acute diarrhoea.

MATERIALS AND METHODS

The study period ranged from 30-7-85 to 20-9-85.The study was conducted at Yangon Children's Hospital and children between the ages of 1 and 35 months presenting with a history of acute diarrhoea of 72 hours or less was taken as diarrhoea cases.Diarrhoea was defined as a sudden in-crease in the frequency of stools (3 times/day) and/or increase in the liquidity of stools. An interval of 24 hours was designated to divide one episode from another. For each diarrhoea case, a child of the same sex, age (± 30 days for cases 1 - 11 months of age; ± 60 days for cases 12 - 35 months of age), socio-economic status, geographic area and ethnic group who has not had diarrhoea within the past month was included as a control. During the study period 58 cases and controls were included in the less-than 12 months age group. The 12-35 months age group consisted of 28 cases and 28 controls.

Collection of stool samples

From each case and control at least 5 ml of faeces was collected after admission. If the child was unable to excrete stool within one hour of admission, a specimen was obtained by insertion of a short lubricated rubber tube into the rectum. Specimens were placed in sterile screw-capped containers.

Bacteriological methods

Bacteriological processing was done within 2 hours. Stools were plated directly onto MacConkey agar, Salmonella shigella (SS) agar, Deoxycholate Hydrogen Sulfide Lactose (DHL) agar (Nissui, Japan), TCBS (Thio-sulphate Citrate Bile salts Sucrose) agar, Vibrio Agar and Blood Agar. In addition, part of each specimen was cultured in enrichment broth of Selenite F and alkaline peptone water, and inoculated onto solid media for isolation after appropriate incubation. All these plates were examined for *Salmonella, Shigella* and *Vibrio* species by standard methods. Salmonella-like and Shigella-like colonies were further characterized biochemically and by serotyping. Vibrio-like colonies were further identified biochemically, and by serotyping and determining their salt tolerance properties.

For *E. coli* isolation, 3 - 10 lactose fermenting colonies with typical *E. coli* morphology were picked from among the colonies grown from each stool sample. The other non-lactose fermenting organisms and suspected pathogens from different media were picked and further characterized by biochemical tests and by serotyping. For identification of Enterotoxigenic *E. coli (ETEC)* as heat labile (LT)toxin strains it was done by Chinese Hamster Ovary cell assay and BIKEN assays. For heat stable (ST) toxin strains it was done by using suckling mice as reported previously by Tin Aye *et al.* (1).

RESULTS

Major bacterial pathogens were isolated from 46.5 per cent and 38.4 per cent of diarrhoeic and control stools respectively. When examined in different age groups it was observed that 37.9 per cent and 64.3 per cent of the pathogens were isolated from less than 1 year age group and from 1 - 3 year age group, respectively (Table 1).The major pathogen responsible for diarrhoea was found to be Enterotoxigenic *E. coli* 30.2 per cent and 9.3 per cent from diarrhoea and control groups respectively and found to be significant between the two groups studied. it was noted that in the diarrhoea group, 12 (14.0 per cent) were ST, 9 (10.5 per cent) were LT and 5 (5.8 per cent) were STLT. In the control group,3 (3.5 per cent) were ST, 4 (4.7 per cent) were LT and 1 (1.2 per cent) were STLT.Thus ETEC was the commonest bacterial agent encountered in this study.

Table 1. Isolation of major pathogens responsible for acute diarrhoea in children (Yangon Children's Hospital)

	< 1 yr		1 - 3 yrs		Total < 1-3 yrs	Total < 1-3 yrs
	Diar (A)	Control (B)	Diar (C)	Control (D)	Diar (A+C)	Control(B+D)
No of cases tested	58	58	28	28	86	86
Enterotoxigenic *E. coli* (ETEC)	16(27.6)	6(10.3)	10(39.3)	2(7.1)	26(30.2)	8(9.3)
ST	8(13.8)	3(5.2)	3(10.7)	0	12(14.0)	3(3.5)
LT	7(12.1)	3(5.2)	2(7.1)	1(3.6)	9(10.5)	4(4.7)
STLT	1(1.7)	0	5(17.9)	1(3.6)	5(5.8)	1(1.2)
Enteropathogenic *E. coli* (EPEC)	5(8.6)	4(6.9)	2(7.1)	2(7.1)	7(8.1)	6(7.0)
Edwardsiella	0	1(1.7)	1(3.6)	0	1(1.2)	1(1.2)
Salmonella	1(1.7)	8(13.8)	0	2(7.1)	1(1.2)	10(11.6)
Shigella sonnei	0	0	1(3.6)	1(3.6)	1(1.2)	1(1.2)
Plesiomonas	0	3(5.2)	1(3.6)	3(10.7)	1(1.2)	6(7.0)
Vibrio	0	0	3(10.7)	1(3.6)	3(3.5)	1(1.2)
	22(37.9)	22(37.9)	18(64.3)	11(39.3)	40(46.5)	33(38.4)

Figures in parenthesis denote percentages.
Diar = Diarrhoea

It was noted that Enteropathogenic *E. coli* (EPEC) and *Edwardsiella* isolation rates did not show significant difference between the two groups studied. It was observed that *Salmonella* isolation rate was higher in the control group (11.6 per cent), than in the diarrhoea group (1.2 per cent) and found to be significant between the two groups studied. *Shigella sonnei* was isolated from the 1 - 3 years age group, one from the diarrhoeal patient and one from the control group.

Plesiomonas was isolated from 1 case of diarrhoea (1.2 per cent) and 6 cases of control (7.0 per cent) and thus it was significantly higher in the control group than in the diarrhoea group. Of the 3 strains or vibrios isolated from diarrhoea cases (3.5 per cent), 2 were *V. cholerae* Inaba and 1 was *V. fluvialis*.

One strain of *V. mimicus* was isolated from the control group (1.2 per cent).

Serotypes of Enterotoxigenic *E.coli* (ETEC) Enteropathogenic *E. coli* (EPEC) and Enteroinvasive *E.coli* (EIEC) were shown in Table 2. Out of 60 strains, 39 strains were

Table 2. Serotypes of *Escherichia coli* isolated

Group	Serotype		A	B	C	D	Total
ETEC (39)	08	H9			2		2
	08	H11				1	1
	023	H-	1				1
	025	H-	2		2		4
	027	H20	1				1
	058	HUT	1				1
	078	H-	1				1
	086	H11	1				1
	086	H18		1			1
	0126	H12	1		3		4
	0126	HUT			1		1
	0128	H27	1				1
	0153	H10	1				1
	0159	H16			2		2
	0159	H34	1		1		2
	OUT	H7	1				1
	OUT	H9	1				1
	OUT	H10	1				1

			A	B	C	D	Total
	OUT	H12	1				1
	OUT	H18	1	1			2
	OUT	H41		2			2
	OUT	H48	1				1
	OUT	HUT		1	2		3
	OUT	H-	1	1		1	3
EPEC (19)	026	H36	1				1
	044	H18		1			1
	055	H27				1	1
	086	H21	1				1
	0111	H4		1			1
	0111	H45		1			1
	0114	H2	1				1
	0114	H32		1			1
	0119	H6	1				1
	0125	H12	1	3			4
	0125	H21		1			1
	0125	H27			1		1
	0125	H-			1		1
	0126	H31		1			1
	0127	HUT		1			1
	0146	H19				1	1
EIEC	0112	H-			1		1
	0124	H-			1		1
Total			23	16	17	4	60

A= diarrhoea < 1 yr B= control < 1 yr
C= diarrhoea 1-3 yrs D= control 1-3 yrs

ETEC, 19 strains were EPEC and 2 strains were EIEC. Among ETEC the serotypes sought were 08H9, H11, 023H-, 025H-, 027H20, 058HUT, 078H-, 086H11, H18,0126H12 HUT,0128H27, 0153H10,0159H16, H34. Among EPEC, the serotypes sought were 026H36, 044H18,055H27, 086H21, 011H4, H45, 0114H2, H32, 0119H6, 0125H12, H21, H-, 0126H31, 0127HUT and 0146H19. Among EIEC the serotypes sought were 0112H- , 0124H-. The serotypes of *Salmonella* and *Shigella* isolated were shown in Table 3.The species included *S. krefeld, S. senftenberg, S. stanley, S. paratyphi B, S. derby* and *S. enteritidis*.

Mixed pathogens isolated from diarrhoea and control cases were shown in Table 4. *Plesiomonas* mixed with other bacteria such as ETEC, or EPEC or *Vibrio* was found in 4 cases 1 from diarrhoea and 3 from

Table 3. Serotypes of *Salmonella* and *Shigella* isolated

Genus	Serotype	A	B	C	D	Total
	paratyphi B		1			1
	stanley	1	2			3
	derby		1			1
Salmonella	*typhimurium*				1	1
	enteritidis		1			1
	krefeld		2			2
	senftenberg		1		1	2
Shigella	*sonnei*			1	1	2

A = diarrhoea < 1 yr
B = control < 1 yr
C = diarrhoea 1 - 3 yrs
D = control 1 - 3 yrs

control cases. *Salmonella* with EPEC was found in 3 of the control cases. *Shigella* mixed with ETEC was found in one and ETEC mixed with EPEC was found in 3 diarrhoea cases. Thus a total of 11 cases had mixed infection, 5 from diarrhoea cases and 6 from the control cases.

Relationship between ETEC and EPEC was shown in Table 5. A total of 16 strains producing ST were serotyped and 4 strains showed 0126 serogroups (25.0 per cent) with different H serotypes (3 were H12 and one untypable H). The other serotypes include 08H9 ,025H-, 027H20, 078H-, 086H11,

Table 4. Mixed pathogens isolated from diarrhoea cases

	< 1 yr Diar A	< 1 yr Control B	1 - 3 yrs Diar C	1 - 3 yrs Control D	Total Diar	Total Control
Plesiomonas + others	0	2	1	1	1	3
Salmonella + EPEC	0	3	0	0	0	3
Shigella + ETEC	0	0	1	0	1	0
ETEC + EPEC	1	0	2	0	3	0

Diar = Diarrhoea

H18,0128H27 and 0153H10. 16 strains producing LT were

Table 5. Relationship between ETEC and EPEC

	< 1 yr		1 - 3 yrs	
	Diar(A)	Control(B)	Diar(C)	Control (D)
ST(16)	025 H-	086 H18	08 H9	
	027 H20	OUT H-		
	078 H-	OUT HUT	0126 HUT	
	0126 H12			
	0128 H27			
	0153 H10			
	OUT H18			
	OUT H48			
LT(16)	023 H-	OUT H18	025 H-(2)	08 H11
	025 H-			
	058 HUT	OUT H41(2)	0159 H34	
	0159 H34			
	OUT H7		OUT HUT	
	OUT H9			
	OUT H12			
	OUT H-			
STLT(6)	OUT H10			OUT H-
			0126 H12	
			0159 H16(2)	
			OUT HUT	

Figure in parentheses denote number of cases detected
Diar = Diarrhoea OUT = O untypable
HUT = H untypable

serotyped, of which 2 strains showed 0159 serogroups with H34 serotypes (12.5 per cent).

The other serotypes identified were 08H11, 022H-, 025H- and 058HUT. 6 strains producing both ST and LT were serotyped and 2 of them showed 0159H16 and the other serotypes identified were 0126H12, 08H9.

DISCUSSION

Diarrhoeal diseases which are often caused by microorganism or their toxins, constitute the greatest mortality and one of the greatest cause of morbidity on a global scale and depending on age and living conditions, average attack rates of diarrhoeal illnesses exceed one to six episodes per person per year and diarrhoea illnesses account for the greatest portion of the staggering childhood mortality and exceed 20 per cent in the poorest areas (2.7). Although the classic secretory enterotoxin of *V. cholerae* causes the most dramatic disease, *E. coli* that produce one or more enterotoxins are the commonest recognized causes of diarrhoea in tropical developing areas (7,8). As indicated from earlier reports ETEC is an important pathogen in tropical and developing countries particularly in infants under 5 years of age(1,2,3,4).From this study also it was noted that 30.2 per cent and 9.3 per cent of ETEC were isolated from diarrhoea and control cases respectively. The higher isolation rate of ETEC as compared to other pathogens indicates that it plays an important role in the causation of diarrhoea in children. It was reported by various authors that 60 per cent of ETEC could be serotyped (9 - 14). In this study it was found that 61.5 per cent of ETEC could be serotyped and the serotypes identified were, 08,023, 025, 058, 078, 086, 0126, 0128, 0153 and 0159. It was also observed that isolation rate of *Salmonella* and *plesiomonas* was higher in the control than in the diarrhoea group.The isolation rate of *Salmonella* and *Plesiomonas* was found to be 11.6 per cent respectively in the control group. *Plesiomonas shigelloides* was the new pathogen identified in this study. it was reported by various workers that *Plesiomonas* was insolated from samples of diarrhoeic stools in subtropical and tropical countries as Mali, Zaire, Kenya, Madagascar, Tahiti, Cuba, India; (15,16), Japan (17); Australia (18) and occasionally Europe and the United States (19). Carriers are quite rare in Europe (19) but was reported that it was more frequently observed in association with epidemics (17,18). The isolation rate of *Vibrio* was 3 per cent in the diarrhoea group and 1 per cent in the control group. 2 of them were found to be *V. cholerae* Inaba and one *V. fluvialis* from the diarrhoea group and they were from 1 - 3 years age group. From the control group also 1 *V. mimicus* was isolated and it was also from 1 - 3 years age group. One of the causes of the control group having a high isolation rate of pathogens might be the fact that in most developing countries the adult foods were heavily contaminated with bacterial pathogens (20). In Myanmar, food, currency notes, flies and water are highly contaminated with enteric pathogens (21,22,23) and may contribute to the dissemination of these pathogens.

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