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The association of enteropathogenic Escherichia coli (EPEC) and gastroenteritis

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A total of 619 cases of adults with gastroenteritis admitted to Yangon General Hospital, Workers' Hospital and Infectious Diseases Hospital were included in this study. It was observed that 33 per cent were suffering from gastroenteritis due to enteropathogenic Escherichia coli (EPEC). The most prominent EPEC serogroups encountered were 01, 026, 055, 086, 0114, 0125 and 0128. The antibiotic sensitivity pattern showed that 87 per cent of enteropathogenic Escherichia coli (EPEC) were resistant to ampicillin, 58 per cent to chloramphenicol, 49 per cent to streptomycin, 55 per cent to benzyl penicillin, 44 per cent and 21 per cent respectively to tetracycline and septrin. They were all sensitive to nalidixic acid but 97 per cent to gentamicin, 96 per cent to sisomycin and 95 per cent to tobramycin respectively.

INTRODUCTION

The term acute infective gastroenteritis or infectious diarrhoea is applied to cases of gastroenteritis with frequent loose stools, with or without vomitting, in whom an infection of the gastrointestinal tract is presumed or known to be the cause. The principal microorganisms causing infective gastroenteritis are shigellae, salmonellae and enteropathogenic strains of Escherichia coli (EPEC). Extensive studies carried out on a world-wide basis have shown that specific bacterial pathogens could be isolated from 22 and 64 per cent of such cases. Thus about 50 per cent could not be diagnosed by conventional bacteriological techniques (Anathan et al., 1989; Regua et al., 1990) [1,2]. E. coli serotypes comprise the largest group of microorganisms inhabiting the intestinal tract. Infection with these strains sometimes occur without symptomatic illness even in the infant.

This study aims (1) to determine the role of EPEC in adults with gastroenteritis; (2) to determine the distribution of serotypes in the adults and (3) to study the antibiotic sensitivity of these strains.

MATERIALS AND METHODS

Study cases

Random samples of 619 cases of adults with gastroenteritis admitted to Yangon General Hospital, Workers' Hospital and Infectious Diseases Hospital were included in this study. These comprised of 409 males and 210 females and their ages ranged from 18 to 65 years. The stool samples were transported in Cary Blair media or in clean containers.

Bacteriological examination of pathogens

It was done according to the method described by World Health Organization manual 1981 [3]. Routinely, MacConkey Agar (MA), Xylose Lysine Dextrose Agar (XLD) and Salmonella-

Shigella agar (SS) were used for the isolation of Enterobacteriaceae. Thioglycolate Citrate Bile Salt (TCBS) Agar was used for isolation of vibrios. For secondary culture, alkaline peptone water and Selenite F broth were used for isolation.

Identification of bacterial pathogens

Biochemical characterization was carried out by using Triple Sugar Iron Agar (TSI), Mortility Sulphide Indole Agar (SIM), Lysine Iron Agar (LIA) or Lysine Indole Motility Agar (LIM). Urease tests, Gram staining, oxidase reaction and other biochemical reactions were done wherever necessary.

Serological testing

For Shigella and Salmonella species, serological tests were done with appropriate antisera available from Denka Seikan Co. Ltd., Japan. For Escherichia coli, the locally prepared pooled polyvalent antisera were used for screening the organisms and further confirmed by specific monovalent antisera. Further confirmation for specific somatic antigen 'O' and flageller antigen 'H' were also done by using homologous antisera.

Antibiotic sensitivity test

The available 21 types of antibiotic discs from BBL, USA and Denka Seikan Co., Ltd., Japan were used for antimicrobial sensitivity testing on Mueller Hinton agar and in broth media. A growth of approximately 10^8 organisms/ml adjusted by MacFarland turbidity tubes were spread on the media and sensitivity test was done using antibiotic discs.

Interpretation for zone size diameters

Interpretative zone standards were performed as described by Barry and Thernsberry, 1985 [4]. These were used to define the interpretive categories in our series.

RESULTS

It is shown in Table 1 that 33.0 per cent of adults were suffering from enteropathogenic E. coli. Out of these patients, 24.5 per cent passed blood and mucous, 1.5 per cent mucous only, 17.2 per cent watery diarrhoea and the remaining 56.9 per cent semisolid type of stool.

Table 2 represents the distribution of serogroups of enteropathogenic Escherichia coli among 204 cases. It is shown that the prevalence of 01 was (6.9) per cent, 026 (8.8) per cent, 055 (10.8) per cent, 086 (6.4) per cent, 0114 (8.8) per cent, 0125 (6.9) per cent, 0126 (5.9) per cent, 0128 (6.7) per cent and 0142 (4.9) per cent. The other serogroups were found to be scattered.

Table 3 shows the resistance pattern to different antibiotics of Enteropathogenic E. coli (EPEC) and resistance to ampicillin was 87 per cent, chloramphenicol 58 per cent, streptomycin 49 per cent, benzyl penicillin 55 per cent, tetracycline 44 per cent and septrin 21 per cent respectively.

It was observed that 77.8 per cent 01 serogroups of E. coli were resistant to ampicillin, 22.2 per cent to chloramphenicol, 33.3 per cent to carbenicillin, 44.4 per cent to penicillin, 55.6 per cent to streptomycin, 55.6 per cent to tetracycline and 11.1 per cent to trimethoprim. They were sensitive to amikacin, furazolidone, gentamycin, kanamycin, neomycin, nalidixic acid, sisomycin and tobramycin (Table 4).

Of the E. coli serogroups 026, 88.9 per cent were resistant to ampicillin. 66.7 per cent to chloramphenicol, 44.4 per cent to carbenicillin, 33.3 per cent to colistin, 11.1 per cent to septrin, 11.1 per cent to sisomycin, 44.4 per cent to streptomycin, 44.4 per cent to tetracycline and 11.1 per cent to trimethoprim respectively. They were sensitive to amikacin,

Table 1. Types of stool in relation to enteropathogenic *Escherichia coli* serogroups (n = 619)

Sr. No.	*E. coli* serogroups	Cases isolated	Types of stool			
			Blood + mucous	Mucous	Watery	Semi-solid
1	01	14	8			6
2	06	5	3		1	1
3	08	7	3			4
4	025	10	1		2	7
5	026	18	3		6	9
6	027	8	1		1	6
7	028	5			1	4
8	044	1				1
9	055	22	3	1	2	16
10	078	2				2
11	086	13	3		2	8
12	0111	2				2
13	0112	2	2			
14	0114	18	4	1	3	10
15	0119	3			1	2
16	0124	1	1			
17	0125	14	2		4	8
18	0126	12	5		3	4
19	0127	2	2			
20	0128	14	3			11
21	0136	1				1
22	0142	10	2	1	2	5
23	0146	2			1	1
24	0148	4	1			3
25	0152	3	1		1	1
26	0157	2			1	1
27	0159	5	1		2	2
28	0164	4	1		2	1
	Total	204	50	3	35	116
	Per cent	33.0	24.5	1.5	17.2	56.9

furazolidone, gentamycin, nalidixic acid and tobramycin.

Of the E. coli serogroup 055, 72.7 per cent were resistant to ampicillin, 72.7 per cent to chloramphenicol, 27.3 per cent to carbenicillin, 36.4 per cent to colistin, 9.1 per cent to kanamycin, 27.3 per cent to penicillin, 18.2 per cent to septrin, 36.4 per cent to streptomycin, 36.4 per cent to tetracycline and 18.2 per cent to trimethoprim respectively. They were sensitive to amikacin, furazolidone, gentamycin, nalidixic acid, sisomycin and tobramycin.

Of the E. coli 0114, 90 per cent were resistant to ampicillin, 50 per cent to chloramphenicol, 30 per cent to carbenicillin, 50 per cent to colistin, 30 per cent to penicillin, 40 per cent to streptomycin and 20 per cent to tetracycline respectively.

They were sensitive to amikacin, furazolidone, gentamycin, kanamycin, neomycin, nalidixic acid, septrin, sisomycin, tobramycin and trimethoprim.

Table 2. Percentage of enteropathogenic *Escherichia coli* by serogroups (n = 204)

Serogroup	Percentage	Serogroup	Percentage	Serogroup	Percentage
01	6.9	086	6.4	0136	0.5
06	2.5	0111	1.0	0142	4.9
08	3.4	0112	1.0	0146	1.0
025	4.9	0114	8.8	0148	2.0
026	8.8	0119	1.5	0152	1.5
027	3.9	0124	0.5	0157	1.0
028	2.5	0125	6.9	0159	2.5
044	0.5	0126	5.9	0164	2.0
055	10.8	0127	1.0		
078	1.0	0128	6.7	Total 204 cases	

Table 3. Resistance pattern to different antibiotics

Sr. No.	Antibiotics	No. of EPEC tested	Resistant cases (per cent)
1	Ampicillin Am2	77	87.01
2	Chloramphenicol C5	77	58.44
3	Carbenicillin CB100	77	28.57
4	Clindamycin CC2	77	98.70
5	Colistin CL10	68	47.05
6	Furazolidone Fx100	77	9.09
7	Penicillin, benzyl P10	44	55.28
8	Streptomycin S10	77	49.35
9	Septrin SXT	68	20.58
10	Tetracycline Te5	77	44.15
11	Trimethoprim TMP5	53	9.09

Table 4. Antibiotic resistance pattern of EPEC serogroups in percentage

Sr. No.	Antibiotics potency	*E. coli* serogroups							
		01	026	055	0114	0125	0126	0128	0142
	n =	9	9	11	10	14	7	9	8
1	Am2	77.8	88.9	72.7	90.0	100.0	85.7	77.8	100.0
2	An30	S	S	S	S	S	S	11.1	S
3	C5	22.2	66.7	72.7	50.0	71.4	42.9	55.6	75.0
4	CB100	33.3	44.4	27.3	30.0	28.6	14.3	11.1	37.5
5	CC2	100.0	100.0	90.9	100	100.0	100.0	100	100.0
6	CF30	NT	NT	NT	NT	7.2	NT	NT	NT
7	CL10	NT	33.3	36.4	50.0	35.7	42.9	66.7	75.0
8	FX100	S	S	S	S	14.3	S	33.3	12.5
9	GM10	S	S	S	S	S	S	11.1	12.5
10	K30	S	NT	9.1	S	7	S	22.2	12.5
11	P10	44.4	NT	27.3	30.0	100.0	NT	NT	NT

Table 4: Contd.

Sr. No.	Antibiotics potency		*E. coli* serogroups							
			01	026	055	0114	0125	0126	0128	0142
		n =	9	9	11	10	14	7	9	8
12	PB300		NT	11.1	NT	NT	NT	S	22.2	12.5
13	N30		S	NT	NT	S	7.1	S	NT	S
14	NA30		S	S	S	S	S	S	S	S
15	NET30		NT	NT	NT	NT	S	NT	NT	NT
16	SXT		NT	11.1	18.2	S	28.6	S	44.4	37.5
17	SIS10		S	11.1	S	S	S	S	11.1	12.5
18	S10		55.6	44.4	36.4	40.0	64.4	57.1	55.6	37.5
19	Te5		55.6	44.4	36.4	20.0	50.0	42.9	66.7	52.5
20	NN10		S	S	S	S	21.4	S	11.1	12.5
21	TMP5		11.1	11.1	18.2	S	21.4	NT	NT	NT

S = Sensitive
NT = Not tested

Of the E. coli serogroup 0125, 100 per cent were resistant to ampicillin, 71.4 per cent to chloramphenicol, 28.6 per cent to carbenicillin, 7.2 per cent to cephalothin, 35.7 per cent to colistin, 14.3 per cent to furozolidone, 7.1 per cent to kanamycin, 100 per cent to penicillin, 7.1 per cent to neomycin, 28.6 per cent to septrin, 64.4 per cent to streptomycin, 21.4 per cent to tobramycin and 21.4 per cent to trimethoprim respectively. They were sensitive to amikacin, gentamycin, nalidixic acid, netilmicin and sisomycin.

Of the E. coli serogroup 0126, 85.7 per cent were resistant to ampicillin, 42.9 per cent to chloramphenicol, 14.3 per cent to carbenicillin, 42.9 per cent to colistin, 42.9 per cent to tetracycline and 57.1 per cent to streptomycin respectively. They were sensitive to amikacin, furazolidone, gentamycin, kanamycin, polymixin B, neomycin, nalidixic acid, septrin, sisomycin and tobramycin.

Of the E. coli serogroup 0128, 77.8 per cent were resistant to ampicillin, 11.1 per cent of amikacin, 55.6 per cent to chloramphenicol, 11.1 per cent to carbenicillin, 66.7 per cent to colistin, 33.3 per cent to furazolidone, 11.1 per cent to gentamycin, 22.2 per cent of kanamycin, 22.2 per cent to polymixin B, 44.4 per cent to septrin, 11.1 per cent to sisomycin, 55.6 per cent to streptomycin and 11.1 per cent to tobramycin respectively. They were sensitive to nalidixic acid only.

Of the E. coli serogroup 0142, 100 per cent were resistant to ampicillin, 75 per cent to chloramphenicol, 37.5 per cent to carbenicillin, 75 per cent to colistin, 12.5 per cent to furazolidone, 12.5 per cent to gentamycin, 12.5 per cent to kanamycin, 12.5 per cent to polymixin B, 37.5 per cent to septrin, 12.5 per cent to sisomycin, 37.5 per cent to streptomycin, 52.5 per cent to tetracycline and 12.5 per cent to tobramycin respectively. They were sensitive to amikacin, neomycin and nalidixic acid.

Table 5 shows that Flagella serotypes were scattered and restricted to some serogroups. Out of 172 cases tested 71 cases (41.2 per cent) were untypable and 13 cases showed no flagella. It is shown that H16 was

Table 5. Serotypes of flagella among different somatic serogroups

Sr. No.	'O' serogroups	Tested	Flagella 'H' type		
			H-	HUT	Others
1	01	14	3	4	H5(2), H15, H16, H21, H32, H48
2	06	5	0	0	H9(2), H16(3)
3	08	7	1	3	H9(2), H21
4	025	10	4	2	H5, H7, H9, H21
5	026	18	1	13	H5, H6, H11, H30
6	027	8	1	4	H11, H17, H21
7	055	22	0	11	H9, H15(2), H17(2), H19, H21, H34, H36, H45, H52
8	078	2	0	1	H16
9	086	13	2	1	H18(3), H19, H21, H30(2), H27, H51
10	0111	2			H31, H45
11	0119	3	0	2	H27
12	0124	1			H16
13	0125	14	0	9	H11, H17(2), H18(2)
14	0126	12	0	7	H10, H15, H18, H25(2)
15	0127	2			H15, H16
16	0128	14	0	6	H2(2), H12(2), H14, H21, H25, H26
17	0142	10	0	4	H6, H15(2), H38, H52(2)
18	0148	4	0	1	H28, H30(2)
19	0157	2	0	1	H6
20	0159	5	0	0	H4(5)
21	0164	4	1	2	H20
	Total	172	13	71	88
	Percent		0.77	41.2	54.2

*Figure in parenthesis denotes number of positive cases

more restricted to 06 serogroup and H4 to 0159 serogroup. It is also shown that 88 cases (51.2 per cent) belonged to different flagella serogroups.

DISCUSSION

In 1985, the role of enteropathogenic E. coli was evaluated in a group of children with endemic diarrhoea admitted to Dhaka hospital and it was reported that 23 per cent of them belonged to serogroups 020, 026 and 018 [5]. Anathan also reported 24.05 per cent of enteropathogenic E. coli were isolated from diarrhoea cases [1]. In Sweden 143 strains of enteropathogenic E. coli of different serogroups were isolated from children with diarrhoea and most of them belonged to serogroups 055, 0119, 0126, 0127 and 0128 [6]. Siitonen reported that E. coli serotypes 01, 02, 06, 07, 018, 025 and Rough-type lipopolysaccharide accounted for 40 per cent of all isolates from 287 healthy adults [7].

Enteropathogenic E. coli strains which were isolated from 18 per cent of children with diarrhoea admitted to the hospital Municipal Salles Neto, Rio de Janeiro were reported to belong to serogroups 0111, 0125, 0126, 0127, 0142, 0128, 0119, 055 and 0114 [2]. In an epidemiological study of enteropathogenic *E. coli* by Olukoya et al. [8], they found that the most common serotype encountered was 055 followed by 026. Katouli et al. [9] also studied the prevalence of different types of diarrhoea-producing *E. coli* in 273 patients attending out-patient clinics in Banda-Abba during 1984 and reported that enteropathogenic *E. coli* belonging to 12 different serogroups of which 0128 and 0126 were the most common were found in 31 per cent of the patients. Sullivan also reported that classical enteropathogenic *E. coli* serotypes associated with diarrhoea were 018, 026, 0119 and 0126 in Gambia children [10]. In out study, out of 619 cases, 204 cases (32.4 per cent) yielded *E. coli* belonging to 28 different serogroups of which 055, 026, 0114, 0128, 0125 and 0126 were most common.

Antibiotics are primarily of value in dysentery. Many other treatments are available, most of which are intended to diminish stool volume, and shorten illness, but none has proven both safe and sufficiently effective to be used routinely in infants and young children [11]. However, no antibiotics or chemotherapeutic agent (including sulfaguanidine, other poorly absorbed sulfonamides, neomycin and halogenated oxyquinolines) has proven to be of value for the routine treatment of acute diarrhoea and their use is inappropriate and possibly dangerous. Olukoya et al., 1988 reported that all strains of enteropathogenic *E. coli* isolated were sensitive to colistin, nalidixic acid, nitrofurantain, cefotoxime, amikacin and augmentin. Of 102 strains, 46 per cent were resistant to one or more of the following antimicrobial agents. Co-trimoxazole, tetracycline, ampicillin, streptomycin, sulfanomides and a combination of ampicillin with sulbactam [8].

Takahashi [12] studied the antibiotic susceptibility of different pathogens and reported that *E. coli* demonstrated the highest level of susceptibility to ofloxacin and relatively high susceptibilities to oxolinic acid, ampicillin, kanamycin and chloramphenicol. In cases studied by Filali et al. [13] all 62 strains of *E. coli* serogroups 078, 01 and 02 were sensitive to colistin, flumequin and gentamycin but few strains were resistant to neomycin, nalidixic acid and trimethoprim and intermediate resistant to nitrofurane, sulfanomide, chloramphenicol, spectinomycin and ampicillin. Gonzalel and Blanco [14] reported that Verotoxic *E. coli* (VTEC) were resistant to antibiotics at higher rates than necrotizing *E. coli* (NTEC) and non VTEC and non NTEC also showed the highest multidrug resistant pattern. In our study, EPEC were also resistant to ampicillin and clindamycin at higher rates. They were also resistant to chloramphenicol, colistin, penicillin, streptomycin and tetracycline in more than 40 per cent of the cases tested. Most of the EPEC were sensitive to amikacin, furazolidone, gentamycin, kanamycin, neomycin, nalidixic acid, sisomycin and tobramycin.

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