The Myanmar Health Sciences Research Journal, Vol. 16, No. 1-3, 2004

Bacterial pathogens isolated from acute diarrhoea cases of children at Yangon Childrens' Hospital

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Rectal swab samples were collected in Cary Blair transport media from 313 cases (male=179; female= 134) of children (less than nine years old) who were admitted to Yangon Children's Hospital during 1999 to 2003. They were processed by standard conventional bacteriological methods. The enteric pathogens isolated were enteropathogenic *Escherichia coli (EPEC)* (23.64%); Shigella spp. (3.5%); Vibrio cholerae (8.31%), and Salmonella typhi (0.32%). Total pathogen isolation rate was as follows: 1999 (20.59%); 2001 (35.79%); 2002 (21.82%) and in 2003 (60%). In other terms EPEC isolation rate increased from 16.18% in 1999; 17.89% in 2001; 10.91% in 2002 and 41.05% in 2003. Vibrio cholerae was not isolated in 1999 but increased beginning from 2000 (7.37%); 2001 (3.64%) to 2003 (17.89%). Antibiotic susceptibility pattern of EPEC has shown that they were resistant to ampicillin, amikacin and tetracycline. Also 66.7% and 93.75% of Vibrio cholerae were resistant to ampicillin in 2001 and in 2003 repectively. Similarly, 50% and 75% of V. cholerae were resistant to tetracycline in 2001 and 2003 respectively.

INTRODUCTION

Diarrhoeal diseases are still a major cause of morbidity and mortality among children in developing countries. In Kenya, epidemiological study in children revealed that bacterial diarrhoea was found in 239 (27.7%) of 862 tested cases comprising EPEC, ETEC and EHEC strains, isolated from 119 cases (13.8%), Salmonella spp. (63 cases, 7.3%) and Shigella spp. (56 cases. 6.5%) and this finding suggests that the main routes of infection may be contaminated drinking water and faecal oral transmission of enteric pathogens [1]. Epidemic diarrhoea caused by enteroaggregative E. coli was reported from south India [2]. Bacteriological quality of weaning food and drinking water given to children of market women in Nigeria had shown that bacterial contamination increases significantly with storage time, and was except for the water samples, significantly higher in foods given to children left at home. Reheated leftover foods also had significantly higher bacterial load than the freshly-cooked food. Coliform count varied significantly with source of drinking water. Poor hygiene standard (inferred from bacterial contamination) was generally observed among mothers weaning <or=2-year-old children, while they were engaged in trading activities in the market, thus exposing their children to high risk of diarrhoea [3]. In Western nations, pathogenic biotypes are sporadically encountered in foodborne gastroenteritis, cholera, dysentery, and chronic ulcerative colitis syndromes are recognized. Escherichia coli has been associated on the average with 2 % of the annual food outbreaks and 5% of total cases. In contrast with Salmonella and

Shigella, significance can be assessed only by fulfillment of Koch's postulates. They reported that 2% of isolates from cheeses involved were toxigenic; 14% were invasive [5]. Thus, this research was conducted to determine the changing pattern of causative pathogens of diarrhoea and antibiotic susceptibility pattern in this region.

MATERIALS AND METHODS

Sample collection

Rectal swab samples from 313 cases of children with diarrhoea who were admitted to Yangon Childrens' Hospital during 1999 to 2003 were collected in Cary Blair transport media and transported to Bacteriology Research Division, Department of Medical Research (Lower Myanmar). The subjects comprised of 179 males and 134 females whose ages ranged from two months to nine years. The samples were collected as follows:

1999: August to December = 68 cases (male = 39; female = 29)
2001: June to September = 95 cases (male = 54; female = 41)
2002: February to September = 55 cases (male = 34; female = 21)
2003: May to September = 95 cases (male = 52; female = 43)

Bacteriological examination

Primary isolation of bacterial pathogens was done by streaking the stool samples onto MacConkey agar (MA), Salmonella-Shigella (SS) agar, Thiosulphate Citrate Bile salt Sucrose (TCBS) agar, and incubating at 37°C overnight. Secondary isolation was done by using Selenite F enrichment at 42°C for 24-48 hours and alkaline peptone water at 37°C for six hours for growth of Salmonella-Shigella and Vibrios respectively. The suspected colonies from selective media were picked, and Gram stained and biochemical tests, catalase production, and oxidase reaction were tested and further confirmed by serology [6].

Serological identification of bacteria

A slide agglutination test was done by reacting with specific "O", "K" and "H" antisera. Antisera used were from Denka Seiken Co.Ltd.,Japan.

Antibiotic susceptibility testing

It was done by the method of Kirby-Bauer (1966). Mueller Hinton agar and broth were used. The zone sizes were measured and interpretation was drawn from the chart given by the disc products [7].

RESULTS

Isolation of bacterial pathogens

Table 1 shows the isolated bacterial pathogens from different studies during the period of 1999 to 2003. The isolation rate was 20.59% in 1999, 34.74% in 2001, 21.82% in 2002, and 60% in 2003.

Pathogens isolated from different age groups
As shown in Table 2, the pathogens isolated were EPEC, Shigella boydii, Shigella dysenteriae, Vibrio cholerae, O1 and O139, Salmonella typhi, Plesiomonas shigelloides and Aeromonas hydrophilia. It also showed the distribution of different pathogens in those periods. Vibrio cholerae was not isolated in the year 1999.

Distribution of enteropathogenic Escherichia coli during 1999-2003

Table 3 shows the distribution of EPEC in different age groups during 1999 to 2003. It was observed that isolation of EPEC was 13.24%, 17.89%, 10.91% and 41.05% in 1999, 2001, 2002 and 2003 respectively. It was found that the isolation rate was more in the age group of 6 months to 3 years.

Table 1. Isolation of bacterial pathogens during 1999 to 2003.

Bacterial species	1999 (n=68)		2001 (n=95)		2002 (n=55)		2003 (n=95)		all group
	M	F	M	F	M	F	M	F	
Klebsiella aerogenes	1	0	0	0	0	0	0	0	1
EPEC	5	6	11	7	2	4	22	17	74(23.64)
Shigellae	1	1	4	1	1	2	1	0	11(3.51)
Plesiomonas shigelloides	0	0	2	0	0	0	0	0	2(0.64)
Aeromonas hydrophilia	0	0	2	0	0	0	0	0	2(0.64)
Vibrio cholerae O1	0	0	4	1	1	1	6	3	16(5.11)
Vibrio cholerae O139	0	0	1	1	0	0	2	6	10(3.20)
Salmonella typhi	0	0	0	0	0	1	0	0	1(0.32)
Total number of isolates	7	7	24	10	4	8	31	26	117(37.38)
Tested cases	39	29	54	41	34	21	52	43	313
Percentages (each sex)	17.95	24.14	44.44	24.39	11.76	38.1	59.62	60.67	
Percentages (both sexes)	20.59		35.79		21.82		60.00		37.38

Specimens collected in 1999: August to December 2001: June to September
2002: February to September 2003: May to September

Table 2. Pathogens isolated from different age groups

Age months	1999-2000 n=68	2001 n=95	2002 n=55	2003 n=95
<6 -6	EPEC (1) *K. aerogenes* (1)	EPEC (2) *A. hydrophila* (1) *P. shigelloides*(1) *S. sonnei* (1) *V. cholerae* O1(1)	EPEC (1)	EPEC (4) *V. cholerae* O1(2)
6-12	EPEC (6)	EPEC (7) *P. shigelloides* (1) *S. dysenteriae* (1) *S. sonnei* (1) *V. cholerae* O139 (2)	EPEC (2) *S. flexneri* (2) *V. cholerae* O1 (1)	EPEC (12) *S. dysenteriae* (1) *V. cholerae* O1 (1) *V. cholerae* O139(1)
12-24	EPEC (3) *S. boydii* (1)	EPEC (8) *A. hydrophilia* (1) *V. cholerae* O1 (1)	EPEC (2) *S. flexneri* (1) *S. typhi*(1)	EPEC (14) *V. cholerae* O1 (2) *V. cholerae*O139 (5)
24-36	nil	*S. dysenteriae*(1) *S. sonnei* (1) *V. cholerae*O1 (1)	EPEC (1)	EPEC (7) *V. cholerae* O1 (3)
36-48	nil	*V. cholerae* O1 (1)	*V. cholerae* O1 (1)	EPEC (2) *V. cholerae* O1 (1) *V. cholerae*O139 (1)
48-60	*S. boydii* (1)	*V. cholerae* O1(1)	nil	nil
>60	EPEC (1)	nil	nil	*V. cholerae* O139(1)
All age group	EPEC (11) *Klebsiella* (1) *S. boydii* (2)	EPEC (17) *A. hydrophilia* (2) *P. shigelloides* (2) *S. dysenteriae* (2) *S. sonnei* (3) *V. cholerae* O1 (5) *V. cholerae* O139(2)	EPEC (6) *S. flexneri* (3) *S. typhi* (1) *V. cholerae* O1 (2)	EPEC (39) *S. dysenteriae* (1) *V. cholerae*O1 (9) *V. cholerae*O139(8)
% isolation	14 (20 59%)	33 (34.74%)	12 (21.82%)	57 (60.00%)

Table 3. Distribution of EPEC during 1999-2003

Age group (months)	1999		2001		2002		2003	
	Male	Female	Male	Female	Male	Female	Male	Female
<6 -6	1/8	0/1	1/8	1/7	0/3	1/4	3/7	1/2
6-12	1/15	5/16	4/20	3/17	1/15	1/10	5/14	7/13
12-24	2/9	1/5	6/18	2/13	1/10	1/4	9/19	5/17
24-36	0/7	0/3	0/5	0/3	0/2	1/1	3/6	4/7
36-48	0	0/1	0	0/1	0/3	0/1	2/5	0/1
48-60	0	0/2	0/1	0	0	0	0/1	0
>60	0	1/1	0/2	0	0/1	0/1	0	0/3
All age group (percent)	4/39 (10.26)	7/29 (24.14)	11/54 (20.37)	6/41 (14.63)	2/34 (5.88)	4/21 (19.05)	22/52 (42.31)	17/43 (39.53)
Both sexes	11/68 (16.18%)		17/95 (17.89%)		6/55 (10.91%)		39/95 (41.05%)	

Distribution of bacterial pathogens in different areas of Yangon (2003)

As shown in Table 4, pathogen isolation ranged from 63.3% to 100% in different areas. From area I (Dagon, Kyauktada & Lanmadaw) all 6-cases admitted to YCH had stool samples from which all had pathogens including 2-cases of *V. clolerae* O139. From area II (Pazundaung & Mingalartaungnyunt) 10 cases out of 15 cases tested (66.67%) had pathogens isolated from their stool including 3-cases of *V. cholerae* O1 and one case of *V cholerae* O139. From area III (Kamayut, Kyimyin-daing, Mayangone & Yankin, out of 10 cases tested, 7-cases were found to be infected with pathogens; including [3] cases of *V. cholerae* O1. From Dallah, Dawpon, Seikkyi and Tharketa, 14 cases out of 23 cases had pathogenic isolates, 2-cases each with *V. cholerae* O1 and O139. From Dagon, Hlaingtharyar, Insein, Shwepyithar, Twante and Thanhlin area, 16 out of 18 cases were infected with pathogens (88.89%) of which one case of *V. cholerae* O1 and 3-cases were *V. cholerae* O139.

Antibiotic susceptibility pattern of pathogens

Table 5 shows that from 39 isolates of EPEC, 92.31% were resistant to ampicillin, amikacin, streptomycin and tetracycline; 76.92% resistant to carbenicillin; 74.36% to trimethoprim/ sulfamethoxazole and 56.41% to chloramphenicol. Comparison of the resistance pattern between *V. cholerae,* EPEC and *Shigella* specie is shown in Table 5. The resistant pattern of pathogens from 2001 to 2003 is also shown. It was found that the resistant pattern of EPEC changed from 10% in 2001 to 97.44% in 2003 with amikacin; 10% in 2001 to 94.87% in 2003 with norfloxacin and 60% in 2001 to 94.57% in 2003 with tetracycline.

DISCUSSION

Enteropathogenic *E. coli* (EPEC) is a common cause of enteritis with colonization of organisms in the duodenum and ileum. Haemorrhagic colitis associated with a rare *Escherichia coli* strain O157:H7 manifesting illness characterized by severe crampy abdominal pain, initially watery followed by grossly bloody diarrhoea, and little or no fever was recognised from two outbreaks in Oregon and Michigan in 1982. They found that it was associated with hamburger meat eaten at restaurants. The attack rate for persons eating sandwiches was estimated to be about 1 case per 1000 sandwiches; 1.8 cases per 1000 for hambergers in those areas. Though the mechanism was not yet known, perhaps by the production of previously unrecognised enterotoxins, it was strongly suggested, but

Table 4. Bacterial pathogens isolated from downtown and outskirts of Yangon (n=95)

Sr. No.	Townships	Isolated pathogens		
		Male	Female	Total
1	Dagon Kyauktada, Lanmadaw	3/3 (100%) O44K74 O119K69 *V. cholerae* O139	3/3 (100%) O125K70 O8K25+*Sh.dysentriae* *V. cholerae* O139	6/6 (100.00%)
2	Pazundaung, Mingala- taungnyunt	6/7 (85.71%) O78K80 O142K+ O144Kx2 *V. cholerae* O1(3)	4/8 (50.0%) O6K15 O148K+ O125K70+O27K+ *V. cholerae* O139	10/15 (66.67%)
3	Kamayut Kyimindaing Mayangone Yankin	6/6 (66.67%) O1K51 O55K59 O111K58 O27K+ + *V. cholerae* O1 *V. cholerae* O1 (2)	1/4 (25.00%) O148K+	7/10 (70.00%)
4	Dala Dawpon Seikkyi Tharkata	8/11 (72.73%) O55K59 O78K80 O78K80+*V. cholerae*O139 O86K62 O128K67 O142K+ *V. cholerae* O1 *V. cholerae* O139	6/12 (50.00%) O86K61 O119K69 O128K67(2) O159K+ *V. cholerae* O1	14/23 (63.63%)
5	Dagon(south) Hlaingtharyar Insein Shwepyithar Tontae Thanhlin	6/7 (85.71%) O26K60 O112K60 O143Kx1 *V. cholerae* O1 *V. cholerae* O139(2)	10/11 (90.91%) O1K51 (2) O8K40 O27K+ O55K59 O86K61 O119K69 O8K25+O159K+ O55K59+O114K90 *V. cholerae* O139	16/18 (88.89%)
6	Other townships	0/18	1/5 O25K+	1/23
	Total	29/52 (55.76%)	24/43 (55.81%)	54/95 (56.84%)

not proven, at that time that it caused the illness, through contaminated meat before cooking or by unknown host susceptibility factors [8]. Study of water sources and environmental transmission of multiple resistant enteric bacteria in rural Bangladesh showed that contamination of water within the household and the wide spread distribution of resistant coliforms in the environment, contributed to the high prevalence of multiple resistant enteric flora in the community [9]. EPEC was isolated as 18.21% of acute diarrhoeic children [10] and 16.1% from dysenteric children in 1996 [11]. Though similar EPEC isolation rates of 16.18% in 1999 and 17.89% in 2001 were obtained; it seemed to increase in the year 2003 (60.00%). *Salmonella typhi* was isolated from one case only in 2002. Though *Vibrio cholerae* was not isolated in 1999, it was isolated from 2001 onwards. The distribution pattern of EPEC and *Vibrio*

Table 5. Resistance pattern of pathogens isolated during 2001 to 2003

Antibiotics (mcg/disc)	EPEC			*Vibrio cholerae*			Shigellae	
	2001 n=10	2002 n=4	2003 n=39	2001 n=6	2002 n=2	2003 n=16	2001 n=4	2002 n=2
Ampicillin, OXOID, Am-10	9	4	36 (92.31)	4	1	15 (93.75)	3	1
Amikacin, BBL, AN-10	1	4	38 (97.44)	1	1	2 (12.50)	1	2
ChloramphenicolBBL, C-30	4	2	23 (58.97)	2	1	6 (37.50)	3	2
Carbenicillin, BBL, CB-100	7	3	30 (76.92)	4	1	9 (56.25)	2	1
Cefalothin,BBL, CF-30	6	3	19 (48.72)	2	0	6 (37.50)	2	1
Gentamicin, BBL, GM-10	1	3	17 (43.59)	1	0	5 (31.25)	1	0
Neomycin, BBL, N-30	3	3	21 (53.85)	0	0	6 (37.50)	1	0
Nalidixic acid, BBL, NA-30	0	NT	15 (38.46)	1	0	6 (37.50)	0	0
Norfloxacin, NOR-10	10	4	37 (94.87)	1	0	3 (18.75)	1	0
Streptomycin, BBL, S-10	10	4	37 (94.87)	4	0	10 (62.50)	3	2
Trimethoprim/ Sulfa-methoxazole, BBL, SXT	6	1	30 (76.92)	4	1	8 (50.00)	1	0
Tetracycline, EIKEN, Te-30	6	3	37 (94.57)	3	2	12 (75.00)	3	2

Figures in parenthesis denote percentages

cholerae were mainly in the children who were from Pazundaung, Mingalataung-nyunt, Dala, Dawpon, Seikkyi, Tharketa, Hlaingtharyar and Shwepyithar areas. The resistant pattern to amikacin also increased from 1.67% in 1996 [10] to 92.31% in 2003 in this study. Similarly, with cephalothin it increased from 9.84% in 1996 [10] to 46.15% in 2003. Thus, it is essential to study the epidemiology, clinical spectrum, and pathogenesis of gastrointestinal illnesses and the reservoir of these agents which are still poorly understood so that gaps that still exist can be elucidated and clarified.

ACKNOWLEDGEMENTS

The authors would like to express their sincere thanks to Professor Dr Paing Soe, Director-General, and Dr. Soe Thein, Deputy Director General, Department of Medical Research, (Lower Myanmar) for their keen interest in research activities; to Dr Tun Pe Director (Research) for his keen interest and encouragement for conducting research, to Medical superintendent, wards in-charge and staff of Yangon Children's Hospital for their kind cooperation.

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