

Antibiotic resistance pattern of enteric bacterial pathogens among childhood diarrhoea

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Antibiotic susceptibility tests on bacterial pathogens isolated from cases of childhood dysentery and diarrhea admitted to some hospitals in Yangon were carried out from 1980 to date. Yearly changing of antibiotic susceptibility pattern was found on these isolates which include enteropathogenic *Escherichia coli* (EPEC), enterotoxigenic *Escherichia coli* (ETEC), *Shigella* species and *Salmonella typhi*. Changing drug resistance pattern of EPEC and ETEC isolates to ampicillin, chloramphenicol and tetracycline was recorded. From this study, it is shown that the drug resistance of *Shigella* species was increased in adults than children. Susceptibility pattern was distinct in newly introduced drugs compared to conventional drugs.

INTRODUCTION

Diarrhoeal diseases constitute one of the causes of morbidity and mortality on a global scale [1]. To an increasingly recognised extent, they are caused by an expanding array of microbial products or "toxins". In Myanmar, diarrhoea and dysentery still play a major health problem and it stood in the fourth position as reported in the National Health Plan (1997-2001) [2]. Acute infectious diarrhoea is caused when a critical number of microorganisms are ingested. They can withstand and survive along the digestive tract even in acidic environment till they reach the intestinal tract. There, they either [i] colonize and multiply in the intestinal lumen or [ii] adhere, penetrate and damage mucosal cells to cause non-inflammatory or inflammatory diarrhoea. Guerrant [3] stated that either in the secretory or in invasive type of diarrhoea, microbial toxins may play critical role in causing diarrhoeal diseases. The magnitude of the global problem of acute diarrhoeal diseases was also reported [4]. Enteric infections caused by *Esch. coli* has been shown since 1971 [5,6]. In 1996,

the outbreak caused by *Esch. coli* O111 was reported [7]. Changes in incidence and causal bacteria in hospital practice were well documented [8, 9, 10]. Antimicrobial resistance of *Salmonella*, *Klebsiella* and *Shigella* was demonstrated and it was quite alarming [11, 12, 13]. Thus, this study was carried out to determine the distribution of enteric pathogens and to test the antibiotic susceptibility pattern on the isolated pathogens in our community.

MATERIALS AND METHODS

The enteric bacterial pathogens were isolated according to the method of WHO [14] and Lennette *et al.* [15] using the products of Difco, Nissui and Oxoid Company Limited.

Isolation of bacterial pathogens were conducted only in the stool samples of children under five years. The collecting sites included Yangon Children's Hospital (YCH), North Okkalapa area and Intakaw village. The stool samples were collected from children with diarrhoea and non-diarrhoea (control) cases in all study sites.

Stool sample collections were conducted throughout the year in YCH but in North Okkalapa area and Intakaw village, collections were made only in the cool-dry and hot-wet seasons as diarrhoea cases occur mainly in these two seasons.

Serotyping of bacterial pathogens was done according to the method of Ewing [16]. To determine labile toxin, verotoxin, adhesive and invasive properties, heat labile test (*in vitro*), verocytotoxic, adhesive, invasive; BIKEN and ELISA tests were done as described by Scotland *et al.* [17]. Antibiotic susceptibility test was done by Kirby-Bauer method [18].

RESULTS

It was observed that ETEC and EPEC were the most common pathogens isolated. Isolated serogroups were O6, O26, O27, O119, O125, O126, O127, O128, O146 and O159 and seemed to be increased from 1996 to 2003.

The antibiotic resistance pattern of enterotoxigenic *Esch. coli* (ETEC) and enteropathogenic *Esch. coli* (EPEC) was changed. In 1980, 25 % of ETEC were resistant to ampicillin and elevated up to 100 % in 1995. Chloramphenicol resistance rose from 26 % in 1980 to 53 % in 1995. Similarly, tetracycline resistance increased from 32 % to 79 %. Regarding EPEC, ampicillin resistance increased from 18 % in 1980 to 89 % in 1996, chloramphenicol from 17 % in 1980 to 62 % in 1996, and tetracycline from 26 % in 1980 to 87 % in 1996 (Table 1).

Antibiotic resistance pattern of *Shigella* species and *Salmonella typhi* isolated from children is shown in Table 2. *Shigella* spp was mostly resistant to ampicillin, chloramphenicol, septrin and streptomycin. Out of 47 strains of *S. typhi*, 88% were resistant to ampicillin, 74% to carbenicillin, 79 % to chloramphenicol and septrin, and 70 % to tetracycline .

Table 1. Changing resistance pattern of enterotoxigenic *Esch. coli* (ETEC) and enteropathogenic *Esch. coli* (EPEC) to antibiotics

Antibiotics/ Antimicrobial agents	Resistance (%)			
	ETEC		EPEC	
	1980 n=99	1995 n= 46	1980 n=82	1996 n=61
Ampicillin AM-10	25	100	18	89
Amikacin AN-30	NT	37	NT	2
Carbenicillin CB-100	NT	NT	NT	80
Cephalothin CF-30	3	0	4	10
Chloramphenicol C-30	26	53	17	62
Colistin CL-10	0	0	12	NT
Furazolidone Fx-100	NT	0	NT	15
Gentamicin GM-10	NT	0	NT	12
Kanamycin K-30	0	NT	6	13
Minocycline MNO-30	NT	NT	NT	16
Nalidixic acid NA-30	NT	0	NT	17
Neomycin N-30	NT	0	NT	NT
Norfloxacin N-10	NT	0	NT	16
Penicillin P-10	93	NT	94	NT
Trimethoprim/sulfamethoxazole (Septrin) SXT	NT	53	NT	80
Sisomycin SIS-10	NT	NT	NT	15
Streptomycin S-10	NT	63	NT	89
Tetracycline TE-30	32	79	26	87

NT= not tested

Table 2. Resistance pattern of *Shigella* and *Salmonella* species isolated from children

Antibiotics / Antimicrobial agents	Resistance (%)	
	<i>Shigella</i> spp. n= 47	<i>S. typhi</i> n=47
Ampicillin AM-10	87	88
Amikacin AN-30	0	5
Cephalothin CF-30	45	21
Carbenicillin CB-100	NT	74
Cefclor Cef-30	NT	43
Ceftriazone CTX-30	NT	19
Chloramphenicol C-30	75	79
Furazolidone Fx-100	36	12
Gentamicin GM-10	7	18
Kanamycin K-30	4	0.5
Minocycline MNO-30	15	NT
Nalidixic acid NA-30	11	11
Netilmicin NET-30	2	3
Neomycin N-30	0	NT
Norfloxacin NOR-10	6	11
Pefloxacin	NT	0
Septrin SXT	75	79
Sisomycin SIS-10	6	NT
Sparfloxacin	NT	0
Streptomycin S-10	75	90
Tetracycline TE-30	66	70

NT= Not Tested

DISCUSSION

This study recorded that bacterial pathogens isolated from different cases were highly resistant to most conventional antibiotics. This being the case, public should take the preventive measures which could avoid the transmission of these causative pathogens. Once personal hygiene and clean environmental conditions are maintained, the occurrence of this infectious disease could be reduced.

According to this study, ETEC and EPEC were found to be the commonest cause of diarrhoea. These were followed by *Shigella* spp. and *Campylobacter jejuni*, the latter being frequently underdiagnosed in the routine microbiology laboratory. *Vibrio cholerae* and *Plesiomonas shigelloides* were sporadic causal organisms for childhood diarrhea.

Some of the diarrhoeal bacterial pathogens were also isolated from asymptomatic controls who did not have diarrhea. Therefore, disease manifestation is due not only to the presence of pathogen, but may also be due to virulent factors of the bacteria, the dosage and the immune status of the host.

Serogroups of *Esch. coli* isolated from children in 1996 and 2003 showed that certain serogroups were increasingly isolated after a period of 7 years. Serogroups O6, O26, O119, O125, O126, O127, O128, O146 and O159 were more prevalent in 2003. There were 14 patients with serogroup O1 in 1996 but, there was none in 2003. One noteworthy result is the isolation of O157 enterohaemorrhagic *E. coli*(EHEC) in both 1996 (2/204) and 2003 (5/532). It is the strain that causes haemorrhagic colitis and even haemolytic uraemic syndrome, a potentially lethal condition.

The antibiotic profile of ETEC and EPEC shows a changing resistance pattern from the year 1980 to 1995. Resistance of ETEC

strains to ampicillin increased from 18% to 100%. Similarly, resistance of EPEC strains increased from 18% to 89%, indicating the virtual ineffectiveness of this antibiotic to *Esch. coli* infections. They were also resistant to chloramphenicol and tetracycline with the same trend. Nowadays both *Shigella* spp. and *S.typhi* were resistant to ampicillin, chloramphenicol and septrin.

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REFERENCES

1. WHO. Management of childhood illness in developing countries. Rationale for an integrated strategy. Integrated Management of Childhood Illness (IMCI). World Health Organization 1999a.
2. National Health Plan (1997-2001). *Planning Document Series 3*, 1997. Union of Myanmar, Ministry of Health; Department of Planning and Statistics.
3. Guerrant RL. Microbial toxins and diarrhoeal diseases: introduction and overview. In: *Microbial toxins and diarrhoeal diseases* 1985; p. 1-13. Pitman. London. (Ciba Foundation Symposium 112).
4. Snyder JD & Merson MH. The magnitude of the global problem of acute diarrhoeal diseases: a review on active surveillance data. *Bulletin of the World Health Organization* 1982; 60: 605-613.
5. Tennant B (eds.). Neonatal enteric infections caused by *Escherichia coli*. *Annals of New York Academy Sciences* 1971; vol:176.
6. WHO. *Escherichia coli* diarrhea, cholera and other vibrio-associated diarrhoea. *Enteric infections due to Campylobacter, Yersinia, Salmonella and Shigella*, WHO/DDC/EPE 79.1, 1980; 80.3, 80.4.
7. Banatvala N, Debeukelaen MM, Griffin PM, Barrett TJ, Greene KD, Green JH & Wells JG. Shiga-like toxin producing *Escherichia coli* O111 and associated hemolytic uremic

- syndrome: a family outbreak. *Paediatric Infectious Diseases* 1996; 15(11): 1008-1011.
8. Watt PJ & Okubadejo OA. Changes in incidence and aetiology of bacteremia arising in hospital practice. *British Medical Journal* 1967; 1:210-211.
 9. Shah M & Watanakunakorn C. Changing patterns of *Staphylococcus aureus* bacteremia. *American Journal of Medical Sciences* 1979; 278: 115-121.
 10. Gedebou M, Tassew A & Azene G. Blood culture isolates from an Addis Ababa hospital frequency and its antibiotic sensitivities. *East African Medical Journal* 1984; 61: 190-196.
 11. Gedebou M. & Tassew A. Antimicrobial resistance and R factor of *Salmonella* isolates from Addis Ababa. *Ethiopia Medical Journal* 1981; 19: 77-85.
 12. Gedebou M. Clinical sources and resistance to antimicrobial agents of *Klebsiella* isolates from Addis Ababa hospital. *Ethiopia Medical Journal* 1982; 20: 109-116.
 13. Gedebou M & Tassew A. *Shigella* species from Addis Ababa: frequency of isolation and *in vitro* drug sensitivity. *Journal of Hygiene* 1982; 47: 77-55.
 14. WHO Manual of diagnostic laboratory procedure for acute enteric infection: simplified methods. World Health Organization, 1983. Geneva.
 15. Leannette EH, Balaws ., Hausler WJ & Shadomy HJ eds. Manual of Clinical Microbiology 1985; American Society of Microbiology, Washington D.C.
 16. Ewing WH. *Edwards and Ewing's Identification of Enterobacteriaceae*. Fourth edition. Elsevier Science Publishing Co.Inc., New York, 1986.
 17. Scotland SM, Gross RJ & Rowe B. Laboratory tests for enterotoxin production, enteroinvasion and adhesion in diarrhoeagenic *Escherichia coli*. In: *Methods for enterovirulence factors*, 1985; p. 395-405. Society for General Microbiology, England.
 18. Bauer AW, Kirby WMM, Sherris JC & Turck M. Antibiotic susceptibility testing by a standardized simple disc method. *American Journal of Clinical Pathology* 1966; 45: 493-496.