

SEROTYPING AND ANTIBIOTIC SENSITIVITY TESTING OF ENTEROBACTERIAL PATHOGENS IN DIARRHOEAL PATIENTS OF KATHMANDU

Subedi A¹, Shrestha C D², Adhikari R P¹

ABSTRACT

In a study carried out at National Public Health Laboratory, Teku, 236 samples were collected from the diarrhoeal patients and half of them were The patients below 15 years of age. Altogether 61 pathogens belonging to the family Enterobacteriaceae were isolated. The presence of pathogens in the patterns below 5 years was highly significant ($P<0.05\%$). E coli was most common (68.8%) followed by Salmonella spp. (16.4%) and Shigella spp. (14.7%). Enteropathogenic was E. coli was the most frequent isolate (71.4%) among the pathogenic E. coli. Similarly, Salmonella typhimurium was the commonest salmonellae and Shigella dysenteriae and Shigella flexneri were the most commonly isolated shigellae. Most of the pathogens were sensitive to Ciprofloxacin (72.1%) and least was sensitive to Ampicillin (9.8%). Only 4.9% of the pathogens were sensitive to all the antibiotics tested.

INTRODUCTION

Diarrhoea is the major cause of morbidity and mortality in the developing countries including Nepal. The causative agents of the infection depend upon the age,⁵ predisposing factors of patients and geographical area. Several members of Enterobacteriaceae are found to be the potent pathogens for causing gastroenteritis and diarrhoea though some of them are considered as the normal flora of gut. Pathogenic E. coli. Salmonella spp.

and shigella spp. are most important members of the family Enterobacteriaceae responsible for causing gastroenteritis and diarrhoea.

Though E. coli is commonly presented as the normal flora in healthy human and animal intestine, its role as a diarrhoea - producing organism became obvious in the late 1940s¹ with the identification of Enteropathogenic E. coli with nursery infection in children. Strains that are primary intestinal pathogens of men are described

1. Central Department of Microbiology Tribhuvan University.

2. National Public Health Laboratory, Teku.

Address for correspondence : Abhignya Subedi
Kha 2-709, Bagbazar, Kathmandu
P.O. Box: 6707, Kathmandu, Nepal.

in four groups^{2,3} as Enteropathogenic *E. coli* (EPEC), Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), and Enterohaemorrhagic *E. coli* (EHEC). As the low numbers of these strains are known to be present in the asymptomatic population, it is also suspected that they produce the disease when they find a favourable condition to develop as the predominant strain in the gut.⁴

Salmonella infections other than enteric fever are called non-typhoid Salmonella infections. The reservoir of the infection is mostly animals and transmitted via contaminated food (faecal oral route). The incident is highest in the summer months and often is severe in the elderly people.⁵

Shigella spp. is the most important cause of dysentery being found in about 60% of all episodes and in nearly all severe episodes; watery diarrhoea may also occur. It is endemic in both tropical and temperate climates and mainly in the places with poor hygienic condition. As very low number as few as 10 organisms are able to cause the infection. Not washing hands properly is one of the main causes associated with childhood dysentery.^{6,7}

In this study, a preliminary examination was made to determine seroprevalence pattern of *E. coli*, *Salmonella* spp. and *Shigella* spp. associated with diarrhoeal cases in Kathmandu and their susceptibility to different antibiotics.

MATERIALS AND METHODS

A total of 236 diarrhoeal stool samples, half from the children below age group 15 years and half from patients above 15 years were collected in the four months of summer season (May - August, 1999) from ORT Ward, Kanti Children Hospital, Gastrointestinal Ward, Shukraraj Tropical Hospital and the outdoor patients in the National Public

Health Laboratory. The samples were collected in transport container according to the manufacturer's instruction, transported to National Public Health Laboratory as soon as possible and cultured with standard microbiological techniques. During the sample collection, patients were asked about their eating habits and the type of antibiotic taken.

A loopful of sample was inoculated into Selenite F broth, MacConkey agar and Desoxycholate Citrate agar and incubated overnight at 37°C. Suspected *E. coli*, *Salmonella* and *Shigella* colonies were identified according to the standard methods⁸ using different tests like: Oxidase, Catalase, O/F, IMViC, reaction in TSI, Urea hydrolysis, Phenylalanine deaminase, Amino acid decarboxylase or dehydrolase, Carbohydrate and related compounds fermentation. They were serotyped using first, with respective polyvalent antisera and then specific monovalent antisera. All the *E. coli* isolates were subcultured in Sorbitol MacConkey agar to identify sorbitol non-fermenting variants of enterohaemorrhagic *E. coli*.⁹

The pathogenic isolates were tested for their susceptibility to seven different commonly used antibiotics viz. Ampicillin (30mg), Chloramphenicol (30mg), Ciprofloxacin (5mg), Cotrimoxazol (25mg), Gentamycin (10mg), Nalidixic acid (30mg), Tetracycline (30mg), by Kirby - Bauer method.

RESULTS

In our study, 54 % of the pathogens were isolated from the patients below the age group 15 years. Among them, the highest rate of isolation was from children below 5 years of age which was greatly significant both among total patients ($\chi^2 = 17.76$ at d.f. = 1) and among children ($\chi^2 = 30.46$ at d.f. = 1).

Out of 465 isolates isolated, 403 were the members of family Enterobacteriaceae and among them, 82 were *E. coli*, 10 were *Salmonella* spp. and 9 were *Shigella* spp. Only 42 (51.2%) out of these 82 *E. coli*. were identified as pathogenic *E. coli*.

The most frequently isolated type of pathogenic *E. coli* was Enteropathogenic *E. coli* (EPEC, 71.4%) followed by Enterohaemorrhagic *E. coli* (EHEC, 11.9%), Enteroinvasive *E. coli* (EIEC, 9.5%) and Enterotoxigenic *E. coli* (ETEC, 7.1%). Among EPEC, serotypes isolated were O1, O18, O26, O55, O111, O119, O125, O127, O128, O142 that of ETEC were, O115 and O159, of EIEC were O124 and O144 and of VTEC were O157.

The number of serotypes isolated is given in table.I.

Table I
Serotypes of *E. coli* isolated

--

Among 10 *Salmonella* spp., the most frequent isolate was *S. typhimurium* (60%) followed by *S. typhi* (20%), *S. enteritidis* (10%) and *S. heidelberg* (10%).

Shigella dysenteriae and *S. flexneri* were most common (44.4% each) among 9 *Shigella* spp. followed by *S. sonnei* (11.1%).

The types and the number of the isolates are given in the table. II.

Table II

Serotypes of *Salmonella* and *Shigella* isolated

--

Most of the pathogens were sensitive to Ciprofloxacin (72.1%), followed by Gentamycin (62.3%), Chloramphenicol (57.4%), Nalidixic acid (47.5%), Tetracycline (36%), Cotrimoxazole (34.4%) and Ampicillin (9.8%). Each type of pathogen showing sensitivity to antibiotics tested is given in the table. III.

Table III : No of pathogens showing sensitivity to different antibiotics tested

--

Na - Nalidixic acid; *Sxt* - Cotrimoxazole;
C - Chloramphenicol; *Amp* - Ampicillin;
Te-Tetracycline; *CN*-Gentamycin; *Cf*-Ciprofloxacin

DISCUSSION

Diarrhoea is generally considered as the infection mostly affecting children. In this study, 54 % of the pathogens were isolated from the patients below the age group 15 years. Among them, the highest rate of isolation was from children below 5 year of age.

In our study, the highest percentage of pathogen isolated was *E. coli*. Though, it is one of the major cause of diarrhoea, it is not tested in the major laboratories as a routine test as done for cholera or shigellosis and only occasionally tested for research purposes.

EPEC was the major isolates (71.43%) in our study. In a study carried out by Aryal (10) in Tribhuvan University Teaching Hospital (TUTH), EPEC was the most common strain (20.2%) whereas, ETEC was the major isolate in the other studies carried out later.^{11,12} Different strains of *E. coli*, has been isolated as the major pathogen causing diarrhoea in children by many researchers from other countries as well, like, India,¹³ Bangladesh,¹⁴ Thailand¹⁵ etc. In most of the cases, its infection is generally considered to be associated with the contaminated water and poor hygienic condition.

S. typhimurium was the commonest isolate (60%) in our study. It was also the most frequent isolate in the other studies carried out in TUTH. 50% of *Salmonella* isolated by Aryal,¹⁰ 75% of it isolated by Gurung¹¹ and 90.9% of it isolated by Shakya¹² were *S. typhimurium*.

S. typhimurium have also been isolated as major species from many parts of the world and most of the infections were considered as food borne.

S. dysenteriae and *S. flexneri* were the commonest species among *Shigella* spp. isolated (44.4% each) in our study. In the other studies carried out earlier in TUTH, *S. dysenteriae* was isolated more frequently than *S. flexneri*.^{11,12,13}

S. dysenteriae and *S. flexneri* are more prevalent in many developing countries of Asia and Africa while; *S. sonnei* is more prevalent in Europe and USA.^{16,17} Infection is found to be associated with the unhygienic behaviours.

Since the use of antibiotics in bacterial diarrhoea is needed, we tested for the sensitivity of different commonly used antibiotics to these isolates.

Most of the *E. coli* was sensitive to Gentamycin

(71.4%) followed by Ciprofloxacin (66.6%) in our Study and 100% of them were resistant to Ampicillin. Aryal,¹⁰ found out that 85.25% of *E. coli* were sensitive to Ciprofloxacin but similar to our, the 100% of the isolates were resistant to Ampicillin. While in the study carried out by Gurung,¹¹ only 22% *E. coli* were sensitive to Gentamycin but interestingly, 100% were sensitive to Ciprofloxacin and to Ampicillin also, 16.7% were sensitive.

For *Salmonella* spp; the drug of choice in our study was Ciprofloxacin to which 80% organisms were sensitive. It is similar to the results of Gurung¹¹ and Shakya¹² who showed 87.5% and 90.3% of sensitivity to this antibiotic respectively.

Similarly, for *Shigella* spp isolated in our study the drugs of choice were Ciprofloxacin to which 88.9% of isolates were sensitive. In the studies carried out previously, 100% isolate were sensitive to Ciprofloxacin.^{11,12}

CONCLUSION

In our study, it was found that children are more affected by diarrhoea than adults. *E. coli* was the major cause of diarrhoea among the patients of Kathmandu and EPEC was the major serotype. *Salmonella* spp. and *Shigella* spp. were also isolated frequently. For *E. coli*, the drug of choice was Gentamycin and for both *Salmonella* spp. and *Shigella* spp. was Ciprofloxacin.

ACKNOWLEDGEMENTS

We would like to thank Mr. Laxman Maharjan, (National Public Health Laboratory. Teku) Dr. Shova Aryal and Dr. J. R. Dhakwa, (Kanti Children Hospital, Maharajgunj) for their generous help during this work.

REFERENCE

1. Hart CA, Batt RM and Saunders JR, Diarrhoea caused by *Escherichia coli*. *Ann-Trop Paediatric* 1993; 13: 121-31.
2. Gross RJ, The pathogenesis of *E. coli*. *Reviews in Medical Microbiology*, 1991; 2:37-44.
3. Raj P, Pathogenesis and Laboratory diagnosis of *E. coli* associated with enteritis. *Clinical Microbiology. Newsletter* 1993; 15: 89-93.
4. Monsur KA, Epidemiology of *Escherichia coli*: An important but neglected field. *J Diarrhoeal Dis Res*. 1985; 3 : 128-133.
5. Sleight JD, *Salmonella*: 2 Bacterial food-poisoning. In: Duguid JP, Marmion BP, Swain RHA, ed. *Mackie & McCartney Medical Microbiology*. Vol 1. Churchill Livingstone. 1983, 320-322.
6. Khan MU, Interruption of Shigellosis by hand washing. *Trans R Soc Trop Med Hyg*, 1982, 76: 164-8.
7. Han AM and Hlaing T, Prevention of diarrhoea and dysenteriae by hand washing. *Trans Roy Soc Trop Med Hyg*, 1989; 83: 128-31.
8. *Bergey's manual of systemic bacteriology* vol. I section 5 p. 12-17 Williams and Wilkins Co. Baltimore 1986.
9. Chapman PA, Siddons CA and Zadik PMJ, An improved selective media for the isolation of *Escherichia coli* O157. *J Med Microbial*, 1991; 35: 107-110.
10. Aryal BK Serological analysis of *Escherichia coli* isolated from various clinical specimens with special interest in gastroenteritis M.Sc. Dissertation (Microbiology), Tribhuvan University, 1996.
11. Gurung C, Study of etiology on acute diarrhoea with special interest in *Salmonella* and *Shigella* M.Sc. Dissertation (Microbiology), Tribhuvan University, 1997.
12. Shakya HR Prospective study on etiology of childhood diarrhoea based clinical features and laboratory investigation. M. Sc. Dissertation (Microbiology), 1999.
13. Deb M, Mohapatra LN, Bhan MK et al., Enterotoxigenic *E coli* diarrhoea in a north Indian Community. *Indian J Med Res*. Sep 1983; 78: 318-23.
14. Moyaenuddin M and Rahaman KM Diarrhoeagenic *E. coli* in hospitalised children in Dhaka, Bangladesh. *Med Res. Counc Bull*, 1985; UI. 11(1): 20-7.
15. Suthienkul O, Sangpetsong V, Sangkasuwan P et al., The prevalence of enterotoxigenic *E. coli* (ETEC) & enteropathogenic *E. coli* (EPEC) in diarrhoeal patients aged under 12. *Bull Fac Med Technol*, Sep. 1983; 7(3).
16. Ling JM, Kam KM, Lam AW and French GL, Susceptibilities of Hongkong isolates of multiply resistant *Shigella* spp to 25 antimicrobial agents including ampicillin plus sulbactam and new 4 quinolones. *Antimicrob Agents Chemother*, 1988; 32: 2-3.
17. Kagalwalla A, Shahid F, Khan N et. al., Childhood Shigellosis in Saudi Arabia. *Paediatr Infect Dis J*, 1992; 11(3): 215-219.

