DECREASED SUSCEPTIBILITY TO ANTIMICROBIALS AMONG *SHIGELLA FLEXNERI* ISOLATES IN MANIPAL, SOUTH INDIA – A 5 YEAR HOSPITAL BASED STUDY

Ballal Mamatha and Chakraborty Rituparna

Department of Clinical Microbiology and Immunology, Kasturba Medical College-International Center, Manipal University, Manipal, Karnataka, India

Abstract. Shigellosis is endemic in many developing countries and an important cause of bloody diarrhea worldwide. Our study was undertaken as a continuation of our earlier study during 2001 - 2006.The aim of this study was to monitor changes in *Shigella* serogroups and resistance patterns to antimicrobials among Shigella isolates. Two thousand one hundred fecal samples were obtained from patients with diarrhea during June 2006 - June 2011. Isolates were identified by standard microbiological techniques as *Shigella* spp and the disk diffusion method was used to determine antimicrobial susceptibility following CLSI guidelines. Of the 2,100 fecal samples, 77 (3.7%) contained *Shigella* spp, of which 73 (94.8%) were *S. flexneri*, 3 (3.9%) were *S. sonnei* and 1 (1.3%) was *S. dysentriae* type 1. *S. boydii* was not identified. One hundred percent resistance was noted against nalidixic acid. There were high levels of resistance to other antimicrobials: ampicillin (100%), Co-trimoxazole (89.6%), tetracycline (84.4%), ciprofloxacin (87%) and norfloxacin (83.1%). Most of the isolates were susceptible to ceftriaxone except for 2 isolates of *S. flexneri.* Antibiotic treatment of shigellosis is needed to prevent mortality. Increasing fluoroquinolone resistance leaves us dependent on third generation cephalosporins for treating shigellosis. Emerging resistance to these cephalosporins was seen in our study.

Keywords: *Shigella*, antimicrobials, decreased susceptibility, India

INTRODUCTION

Shigellosis is responsible for morbidity and mortality in high risk populations, such as children under five years of age, senior citizens, those affected by war and famine and patients with chronic diseases such as HIV, especially in developing countries (Bastos and Loureiro, 2011). *Shigella* spp, are highly infectious, even at low counts. Shigellosis is a major source of gastroenteritis in the world and severe infections may require antimicrobial treatment (Folster *et al*, 2011). However, emergence of multidrug resistance among *Shigella* spp has made selection of empiric antimicrobial therapy difficult (Pickering *et al*, 2009). *Shigella* spp are known for their multidrug resistance which may result from selection of resistant mutants

Correspondence: Dr Mamatha Ballal, Department of Clinical Microbiology and Immunology, Kastuba Medical College-International Center, Manipal University, Manipal 576104, Karnataka, India.
Tel: +91 820 2933019; Fax: +91 820 2571908
E-mail: mamatha_98@yahoo.com

through widespread use of antimicrobials (Mamatha *et al*, 2007). Fluoroquinolone resistance has risen among *Shigella* spp, especially in Asia (Pu *et al*, 2009; Folster *et al*, 2011; Ghosh *et al*, 2011).

The present study is a continuation of an earlier study (Mamatha *et al*, 2007). Our objectives were to monitor changes in serotypes and resistance pattern among *Shigella* spp found in dysentery patients during 2006-2011. These results will be compared with our previous survey during 2001-2006.

MATERIALS AND METHODS

Study population

From June 2006 to June 2011, 2,100 fecal samples were received from patients with diarrhea seen at Kasturba Hospital, Manipal, India, a 1,600 bed tertiary care hospital and sent for culture and sensitivity. The samples were obtained from patients of all ages.

Sample collection and evaluation for enteric pathogens

Fresh fecal samples were received in the laboratory within half an hour of collection and processed using standard microbiological methods (Winn *et al*, 2005). The samples were macroscopically examined for blood and mucus. Microscopic examination was performed to look for white blood cells (WBCs), red blood cells (RBCs), macrophages and ova and cysts of parasites. The samples were directly plated onto MacConkey agar, xylose lysine deoxycholate agar and inoculated into selenite F broth for enrichment and incubated overnight at 37ºC. Colonies morphologically resembling *Shigella* were identified using a battery of standard biochemical tests and further confirmed through serotyping using polyvalent and monovalent type specific antisera (Remel Europe Dartford, UK).

Determination of antimicrobial susceptibility

Susceptibilities of *Shigella* isolates to various antibiotics were determined by the Kirby Bauer's disk diffusion technique following Clinical Laboratory Standards Institute'(CLSI) guidelines. Antimicrobials tested (concentration per disk in g) were: ampicillin (10), tetracycline (30), nalidixic acid (30), ciprofloxacin (5), norfloxacin (10), gentamicin (10), amikacin (10), Co-trimoxazole (25) and ceftriaxone (30). Zones of inhibition were measured in millimeters and compared to those for *Escherichia coli* ATCC 25922 (Colindale, London, UK) which served as the control strain in our study.

The clinical details of the patients were noted wherever applicable and included age, sex, type of ward, presenting clinical features and underlying illnesses.

Ethical considerations

Our study was approved by the ethics committee of Kasturba Hospital.

RESULTS

Of the 2,100 fecal samples, 77 (3.7%) were positive for *Shigella* species: *S. flexneri* (73, 94.8%), *S. sonnei* (3, 3.9%) and *S. dysenteriae* type 1 (1, 1.3%). No *S. boydii* isolates were found during the study. The *S. sonnei* and *S. dysenteriae* type 1 isolates were found during June 2006 and June 2007, respectively. *S. flexneri* with the most common serogroup and 2a was the most prevalent subtype. *Shigella* was isolated from patients aged 4 to 75 years. Of the 77 Shigellae positive cultures, 6 (7.8%) were from children aged 4-6 years and the rest were isolated from adults. Males outnumbered females in all age groups.

Table 1
Comparison of antimicrobial resistance patterns of *Shigella flexneri* between the present study and a previous study

Antibiotics	April 2001-April 2003 *n*=30	May 2003-May 2006 *n*=38	June 2006-June 2011 *n*=77
Ampicillin	19 (63.3%)	24 (63.1%)	77 (100%)
Tetracycline	20 (66.7%)	28 (73.7%)	65 (84.4%)
Co-trimoxazole	20 (66.7%)	30 (78.9%)	69 (89.6%)
Nalidixic acid	17 (56.7%)	30 (78.9%)	77 (100%)
Ciprofloxacin	9 (30.0%)	11 (28.9%)	67 (87.0%)
Norfloxacin	0 (0.0%)	7 (18.4%)	64 (83.1%)
Gentamicin	12 (40.0%)	27 (71.0%)	31 (40.2%)
Amikacin	13 (43.3%)	17 (44.7%)	4 (5.2%)
Ceftriaxone	0 (0.0%)	0 (0.0%)	2 (2.6%)

Table 1 shows a comparison of the resistance patterns in this study with our previous study (Mamatha *et al*, 2007). All the isolates were resistant to two or more drugs. One hundred percent resistance was seen against ampicillin and nalidixic acid. Resistance was also seen against Co-trimoxazole (89.6%), ciprofloxacin (87%), norfloxacin (83.1%), tetracycline (84.4%) and gentamicin (40.2%). The least resistance was seen against amikacin (5.2%). Ninety-seven point seven percent of strains were susceptible to ceftriaxone; resistance was observed in 2 isolates.

The *S. sonnei* and *S. dysenteriae* type 1 isolates were susceptible to nearly all antimicrobials tested except nalidixic acid.

DISCUSSION

In this study, we determined the serotype switch and antimicrobial resistance patterns among *Shigella* spp isolated from patients attending Kasturba Hospital, Manipal, south India from June 2006 to June 2011. *Shigella* spp were found in 3.7% of isolates, which was fewer than our previous study (5.7%) (Mamatha *et al*, 2007) as well as studies from other parts of India which isolation rates of 5% (Taneja *et al*, 2006) and 5.4% (Srinivasa *et al*, 2009), respectively. *S. flexneri* was the most common serogroup isolated (94.8%) and 2a was the most prevalent subtype. This is similar to our previous study (Mamatha *et al*, 2007) and other studies from India (Srinivasa *et al*, 2009; Ghosh *et al*, 2011). The isolation rate of *S. sonnei* decreased by 27.2% from our previous study. *S. dysenteriae* type 1 isolates decreased by 6.7% and *S. flexneri* isolates increased by 49.8% compared to our previous study during 2001-2006 (Mamatha *et al*, 2007).

In India antimicrobial resistance among the genus *Shigella* is more common than among any other enteric bacteria (Taneja *et al*, 2004; Ghosh *et al*, 2011). Due to the emergence of nalidixic acid resistant isolates throughout the world during the 1990s, fluoroquinolones, especially ciprofloxacin, have been used to treat shigellosis. Fluoroquinolone resistance among *Shigella* spp was first reported

in *Shigella dysenteriae* type 1; since then fluoroquinolone resistance has increased in developing countries and also among other serogroups of *Shigella* (Ghosh *et al*, 2011).

All *Shigella* isolates were resistant to nalidixic acid. The other isolates had a marked increase in resistance compared to our previous study (Mamatha *et al*, 2007): resistance to ciprofloxacin increased from 30.0% to 87.0%, norfloxacin from 20.0% to 83.1%, ampicillin from 63.3% to 100%, tetracycline from 73.7% to 84.4% and Co-trimoxazole from 78.9% to 89.6%. However, gentamicin and amikacin had a decrease in resistance from 71.0% to 40.2% and 44.7% to 5.2%, respectively. Shigellae may be susceptible to the aminoglycosides *in vitro*, but have poor penetration of the intestinal mucosa when given orally (WHO, 2005). Most of our isolates continued to be susceptible to third generation cephalosporins, similar to our previous study (Mamatha *et al*, 2007); two isolates were found to be resistant. This may represent an emergence of cephalosporin resistance by Shigella. Similar findings were reported from Bangalore (Srinivasa *et al*, 2009), Puducherry (Mandal *et al*, 2010) and Chandigarh (Taneja *et al*, 2012).

Apart from some fluoroquinolones, pivmecillinam (amdinocillin pivoxil) and ceftriaxone are currently the only antimicrobials effective in the treatment of multidrug-resistant *Shigella* in all age groups. Azithromycin is considered an alternative treatment among adults. Use of these alternative drugs is limited by high cost (pivmecillinam, azithromycin), rapid development of resistance (azithromycin), their formulation (injectable for ceftriaxone, four times a day for pivmecillinam), and limited data on efficacy (ceftriaxone, azithromycin). They should only be used when local strains of *Shigella* are known to be resistant to ciprofloxacin (WHO, 2005).

S. flexneri was the most common *Shigella* serotype in our study. The percent of multidrug resistant strains of *S. flexneri* has increased. Only a few *Shigella* isolates were resistant to the third generation cephalosporin, ceftriaxone. More data from other parts of the country is required to further evaluate these findings. Surveillance is needed in order to implement timely interventions, and limit the spread of multidrug resistant clones. Strict infection control practices, a judicious use of antibiotics by clinicians may help minimize the selection pressure of antibiotic resistance among bacteria.

REFERENCES

Bastos FC, Loureiro ECB. Antimicrobial resistance of *Shigella* spp. isolated in the State of Para, Brazit. *Rev Soc Bras Med Trop* 2011; 44: 607-10.

Clinical and Laboratory Standards Institute (CLSI).Performance standards for antimicrobial disk susceptibility tests. Approved standard. 10th ed. Supplement M02 - A10. Wayne, PA: CLSI, 2011.

Folster JP, Pecic G, Bowen A, Rickert R, Carattoli A, Whichard JM. Decreased susceptibility to ciprofloxacin among *Shigella* isolates in the United States, 2006 to 2009. *Antimicrob Agents Chemother* 2011; 55: 1758-60.

Ghosh S, Pazhani GP, Chowdhury G, *et al*. Genetic characteristics and changing antimicrobial resistance among *Shigella* spp. isolated from hospitalized diarrheal patients in Kolkata, India. *J Med Microbiol* 2011; 60: 1460-6.

Mamatha B, Pusapati BR, Rituparna C. Changing patterns of antimicrobial susceptibility of *Shigella* serotypes isolated from children with acute diarrhea in Manipal, South India, A 5 year study. *Southeast Asian J Trop Med Public Health* 2007; 38: 863-6.

Mandal J, Mondal N, Mahadevan S, Parija

SC. Emergence of resistance to third-generation cephalosporin in *Shigella*– a case report. *J Trop Pediatr* 2010; 56: 278-9.

Pickering LK, Baker CJ, Kimberlin DW, Long SS, eds. *Shigella* infections. Elk Grove Village, IL: American Academy of Pediatrics, 2009: 737-40.

Pu XY, Pan JC, Wang HQ, Zhang W, Huang ZC, Gu YM. Characterization of fluoroquinolone-resistant *Shigella flexneri* in Hangzhou area of China. *J Antimicrob Chemother* 2009; 63: 917-20.

Srinivasa H, Baijayanti M, Raksha Y. Magnitude of drug resistant shigellosis: A report from Bangalore. *Indian J Med Microbiol* 2009; 27: 358-60.

Taneja N. Fluoroquinolone-resistant *Shigella flexneri*: a new therapeutic challenge. [Abstract]. 8th Commonwealth Congress on Diarrhea and Malnutrition (CAPGAN) 2006, Scientific Session 3: Management of Diarrheal Diseases I. ICDDR,B Periodicals, 2006.

Taneja N, Mohan B, Khurana S, Shama M. Antimicrobial resistance in selected bacterial enteropathogens in north India. *Indian J Med Res* 2004; 120: 39-43.

Taneja N, Mewara A, Kumar A, Verma G, Shama M. Cephalosporin-resistant *Shigella flexneri* over 9 years (2001 - 09) in India. *J Antimicrob Chemother*, 2012 (E pub ahead of print on March 10, 2012).

Winn Jr W, Allen S, Janda W, *et al*. Koneman's color atlas and textbook of diagnostic microbiology. 6th ed. Philadelphia: Lippincott Williams and Wilkins, 2005: 213-251.

World Health Organization (WHO). Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. Geneva: WHO, 2005. [Cited 2012 Jul 17]. Available from: URL: http://whqlibdoc.who.int/publications/2005/9241592330.pdf