CHANGING PATTERNS OF ANTIMICROBIAL SUSCEPTIBILITY OF *SHIGELLA* SEROTYPES ISOLATED FROM CHILDREN WITH ACUTE DIARRHEA IN MANIPAL, SOUTH INDIA, A 5 YEAR STUDY

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Abstract. This study was carried out to determine the current pattern of *Shigella* serogroups and their antimicrobial resistance in children with acute gastroenteritis in Manipal, South India. A total of 1,200 stool samples were collected from April 2001 to May 2006 in children suffering from acute gastroenteritis attending the out-patient department of pediatrics at Kasturba Hospital, Manipal, South India. These samples were cultured for enteric pathogens. The isolates were confirmed to be *Shigella* by biochemical reactions and slide agglutination tests using specific antisera. Antimicrobial susceptibility was performed using an agar diffusion technique method following the National Committee for Clinical Laboratory Standard guidelines. Of 1,200 stool samples, 68 (5.6%) were positive for *Shigella* spp, 31 (45%) were *Shigella flexneri* followed by *S. sonnei* in 20 (31%), *S. boydii* in 10 (15%), and *S. dysenteriae* in 6 (8%). Of the 68 isolates, 58 (85.7%) showed resistance to various drugs and 47 (70%) were resistant to two or more drugs. Resistance to trimethoprim-sulfmethoxazole, tetracycline, nalidixic acid and ampicillin was observed in this study. All the strains were resistant to nalidixic acid (100%) but sensitive to cefotaxime and ceftriaxone.

INTRODUCTION

Infectious diarrheal diseases are responsible for considerable morbidity and mortality, especially in developing countries. Dysentery has been a major disease in the history of mankind and one of the commonest killers in the pediatric age group throughout the world (Guerrant *et al*, 1990). *Shigella* accounts for a significant proportion of bacillary dysentery in many tropical and subtropical countries, like India. Attack rates ranging from 1 to 15 % have been reported from various parts of the country. Various species of *Shigella* have been a common cause of diarrhea in Manipal and in other parts of the country. Changing trends in the epidemiology of shigellosis and antimicrobial resistance patterns of *Shigella* isolates have been noticed in many parts of the world over the past two decades (Nyogi *et al*, 2000). Although rehydration therapy is the treatment of choice for acute diarrhea, antimicrobial agents are indicated for the treatment of suspected shigellosis. *Shigella* strains are particularly noted for their multidrug resistance, which may result from the selection of resistant mutants through the wide-spread use of antimicrobial agents. Multidrug resistance in *Shigella* species has been reported from different parts of India. The changing patterns of antimicrobial susceptibilities among *Shigella* isolates makes it difficult to recommend a drug of choice for shigellosis (Jesudason *et al*, 1985; Jadhav *et al*, 1996).

The present study was therefore undertaken to study the changing patterns of *Shigella* serogroups in cases of dysentery over a

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5-year period and to determine the drug resistance patterns and compare the results of the present study with that of previous years with regard to the prevalence of serogroups and antimicrobial sensitivity of the *Shigella* isolates.

MATERIALS AND METHODS

All fecal samples from children with acute diarrhea received for culture of enteric pathogens from both inpatients and outpatients at the Kasturba Hospital, Manipal from April 2001 to May 2006 were included in the study. No transport medium was employed as fresh feces were received in the laboratory within half an hour of collection and processed without delay using standard microbiological methods (National Committee for Clinical Laboratory Standards, 1997). During the study period, a total of 1,200 stool samples were collected from pre-school children (<5 years) and school age children (6-15 years) with acute gastroenteritis attending the out-patient department of Kasturba Hospital, a 1,500 bed tertiary care hospital in Manipal, southern India.

Bacteriological analysis

The samples were examined macroscopically for the presence of mucus and blood and microscopically for white blood cells (WBC), red blood cells (RBC), macrophages, cysts and ova of parasites. The samples were inoculated directly onto MacConkey agar, Deoxycholate Citrate Agar (DCA); enrichment took place in Selenite F broth which was incubated overnight at 37ºC. After enrichment, subcultures were taken from the above media and further incubated overnight at 37ºC. Colonies morphologically resembling *Shigella* were further identified by biochemical reactions and confirmed by slide agglutination test using polyvalent and monovalent antisera from Murex Diagnostics Ltd, England.

Antimicrobial susceptibility testing

Antibiotic susceptibility of the *Shigella* isolates to various antibiotics were determined by the disc diffusion technique of Kirby Bauer and according to the standard procedure outlined in the National Committee for Clinical Laboratory Standard guidelines (Bauer *et al,* 1966; NCCLS, 1997). Antibiotics tested (concentration per disc in µg) were amoxicillin (30), amikacin (10), tetracycline (30), nalidixic acid (30), ciprofloxacin (5), gentamicin (10), norfloxacin (10), cotrimoxazole (25), cefotaxime (30), ampicillin(10), chloramphenicol (30), and ceftriaxone (30).

The patient's demographic details were noted where applicable. These include age, sex, and ward/OPD, presenting clinical features and underlying illness. Zones of inhibition were recorded in millimeters and were compared with those of *Escherichia coli* ATCC 25922 from Colindale, London which served as a control strain.

RESULTS

Shigella was isolated from 68 (57%) of 1,200 stool samples. Of the 68 children, 41 (60.2%) were below 5 years of age and the remaining 27 (39.7%) were between 6-15 years of age. The male to female ratio was 3:1. For convenient observation of changing trends in the *Shigella* spp, our study was divided into 2 parts; the first showing our observations between 2001-2003 and second between 2003-2006. We present here these observations which highlight the trends and changes in *Shigella* serogroups and their susceptibility patterns.

During 2001-2003, *S. flexneri* (45%) was the commonest isolate of the *Shigella* species, followed by *S. dysenteriae* (29.4%), *S. boydii* (14.7%), and *S. sonnei* (10.2%). This profile is in sharp contrast with that obtained before 1995 when *S. dysenteriae* 1 predominated over *S. flexneri*. Our study also shows that among *S. flexneri* strains, *S. flexneri* 2a (35%) was the most common serotype, followed by

Table 1
The percentage isolation of *Shigella* spp between 2001-2006.

Name of the organism	Percentage of isolation (%)	
	2001-2003 n= 30	2003-2006 n=38
Shigella flexneri	14 (45%)	17 (45%)
Shigella dysenteriae	9 (29%)	3 (8%)
Shigella boydii	4 (15%)	6 (15%)
Shigella sonnei	3 (10%)	12 (31%)

Table 2
The percentages of resistance among *Shigella* spp to various antimicrobial agents.

Antimicrobial	Percentage of resistance (%)	
	2001-2003 n=30	2003-2006 n=38
Ampicillin	19 (64%)	24 (64%)
Amoxicillin	16 (55%)	20 (52%)
Amikacin	13 (45%)	17 (45%)
Tetracycline	20 (68%)	28 (75%)
Nalidixic acid	17 (58%)	30 (100%)
Ciprofloxacin	9 (30%)	11 (30%)
Gentamicin	12 (39%)	27 (70%)
Norfloxacin	0 (0%)	7 (20%)
TMP-SMX	20 (68%)	30 (81%)
Chloramphenicol	16 (56%)	24 (64%)
Cefotaxime	0 (0%)	0 (0%)
Ceftriaxone	0 (0%)	0 (0%)

TMP-SMX = Trimethoprim-sulfamethoxazole

3a, 6 and 2b. All strains isolated during this period were uniformly susceptible to norfloxacin, but more than 90% of strains were resistant to tetracycline and trimethoprim-sulfamethoxazole (TMP-SMX) and 64% of strains were resistant to ampicillin. Resistance to amoxicillin, chloramphenicol and nalidixic acid was found in 55%, 46% and 29% of strains, respectively.

Observations during 2003-2006 showed a dramatic change in the *Shigella* serogroups. *S. flexneri* (45%) was the commonest isolate, followed by *S. sonnei* (31%), *S. boydii* (15%), and *S. dysenteriae* (8%). A change in resistance was also observed. All the isolates were sensitive to cefotaxime and ceftriaxone and showed variable resistance against the remaining antibiotics. Resistance to nalidixic acid was highest (100%) followed by Co-trimoxazole (80.7%), tetracycline (74.7%), gentamicin (70%), ampicillin (64%), and chloramphenicol (62%). Ciprofloxacin and norfloxacin had the least resistance. Further analysis revealed that nearly 70% of isolates were resistant to two or more antibiotics.

DISCUSSION

Shigellosis occurs both in epidemic and endemic forms in children and is a major public health problem in developing countries. Over the past decades, *Shigella* spp have become progressively more resistant to most of the widely used and inexpensive antimicrobials. Changes in the incidence of *Shigella* serogroups also makes it difficult to formulate a drug of choice for shigellosis (Bennish *et al*, 1992; Dutta *et al*, 2002).

In the present study *Shigella flexneri* was the predominant serogroup followed by *S. sonnei*. Our observations regarding the changing trends in *Shigella* serogroups correlate well with studies from other parts of India and also other developing countries (Jesudason *et al*, 1985; Hoge *et al*, 1998; Taneja *et al*, 2003). *Shigella flexneri*, the commonest isolate, showed a high degree of resistance to most commonly used drugs, such as ampicillin, TMP-SMX, tetracycline, and nalidixic acid. In our study a rapid increase in the resistance of *Shigella flexneri* to TMP-SMX was noted between 2001 (62%) and 2006 (92%) ($p<0.001$). We observed a drastic increase in resistance among the isolates to nalidixic isolates from 78% in 2001 to 100% in 2006. This may be due to the extensive use of quinolones such as nalidixic acid and the newer fluoroquinolones. An increasing trend in nalidixic acid resistance among *Shigella* spp was

also reported in Kolkata (Dutta *et al*, 1998). Most of our isolates continued to be susceptible to norfloxacin and ciprofloxacin. Although the safety of fluoroquinolones in young children is controversial, several reports about their safe usage in childhood have been published (Schaad *et al*, 1995; Thirunarayanan *et al*, 1997; Cheasty *et al*, 2002). Nalidixic acid is the drug recommended for the empiric treatment of bacillary dysentery in India, but given our present findings its use in patients with shigellosis should be reconsidered. Overall, *Shigella* strains showed a statistically significant increase in resistance to ampicilin, tetracycline, nalidixic acid and TMP-SMX ($p<0.05$) during our study. This indicates a decreased efficacy of these drugs in the empiric treatment of shigellosis in Manipal. We suggest that periodic monitoring of the susceptibility patterns of these *Shigella* stains is important and should be undertaken in other centers so that larger multicentric data are available to study the emergence of drug resistance to commonly used drugs and for choosing appropriate antimicrobial therapy for these infections.

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