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RESEARCH ARTICLE

Surveillance of changing antimicrobial resistance pattern in *Shigella* in North India

***Prabhav Aggarwal, Beena Uppal, Roumi Ghosh, Arun Kumar Jha and Dipasri Konar**
Department of Microbiology, Maulana Azad Medical College New Delhi, India

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****Corresponding Author***

Prabhav Aggarwal

Abstract

Diarrhea and dysentery are responsible for immense morbidity and mortality in developing countries, with *Shigella spp*. being one of the common bacterial causes. Rising trends in antimicrobial resistance rates pose a significant problem in treatment. Therefore, this study was undertaken to analyze the current situation *in vitro* drug resistance of *Shigella spp*. Stool samples were received from 7377 patients of all age groups presenting with signs and symptoms of diarrhea or dysentery from 1st January 2009 to 31st December 2012. Causative organisms were identified by routine microscopic examination, culture techniques and serotyping, followed by susceptibility testing according to CLSI guidelines. *Shigella spp.* were isolated in 143/7377 (1.94%) cases with following distribution: *S. flexneri* (89/143, 62.2%), *S. boydii* (36/143, 25.2%), *S. sonnei* (22/143, 15.4%) and *S. dysenteriae* (2/143, 1.4%). 119 (83.2%) strains were multidrug resistant to three or more agents. High resistance rates were seen for cotrimoxazole (90.9%), nalidixic acid (90.9%) doxycycline (88.8%), ofloxacin (56.6%) and ciprofloxacin (53.8%). Resistance to third generation cephalosporins was 18.9-20.3%. All strains were susceptible to piperacillin-tazobactam combination and carbapemens. Antimicrobial agents like sulphonamides, tetracyclines, amplicillin, cotrimoxazole and nalidixic acid have been popular choices in past. Current drug of choice is ciprofloxacin, however more than half of the strains showed resistance. The third generation cephalosporins are being used with increasing frequency in such cases resulting in emergence of significant resistance to this class as well. Current scenario suggests change in the drug of choice from ciprofloxacin to oral third generation cephalosporin for severe cases.

Introduction

Diarrhea and dysentery are often associated with considerable morbidity and mortality in all the age groups, especially among children. WHO defines diarrhea as the passage of three or more loose or liquid stools per day, or more frequently than is normal for the individual (WHO, 2013a) and dysentery as any diarrheal episode in which the loose or watery stools contain visible blood (WHO, 2013b). Their importance is emphasized by the fact that diarrhea is the second most common cause of death among children under five globally, following closely behind pneumonia. WHO has reported that India has more number of deaths due to diarrhea than any other country in the world (UNICEF/WHO, 2009). There are innumerable agents that are responsible for both these disease spectrum. However, *Shigella* is the most common cause of bacillary dysentery. It has been reported that *Shigella* may be responsible for 9.1% to 22% of the acute diarrheal illness (Kotloff KL et al, 1999) and almost half of the dysentery cases (Khan A et al, 2005). Kotloff KL et al, 1999 reviewed articles published between 1966 and 1997 on *Shigella* infection and reported high global burden of shigellosis. The number of shigellosis cases in developing countries

was estimated to be 163.2 million out of the global burden of 164.7 million cases, signifying huge disparity between the developing and developed regions. India, being a developing country suffers from large number of shigellosis cases.

Treatment of shigellosis with antimicrobial agents is recommended (David KV et al, 2010), which results in reduction in the duration, severity and associated morbidity and mortality. Appropriate treatment of shigellosis depends on correct identification of the resistance patterns. Rapidly emerging resistant *Shigella* strains warrant the need for continuous monitoring of sensitivity patterns and the choice of empirical therapy should be governed by periodically updated local antibiotic sensitivity patterns. Several of the antimicrobial agents that were previously useful, such as sulphonamides, tetracycline, ampicillin and trimethoprim-sulfamethoxazole, have largely become obsolete. Ciprofloxacin, which was a reserve drug previously, has now been recommended by WHO as the drug of choice (WHO, 2005). However, resistance to ciprofloxacin has also developed rapidly, which may very soon become useless. This will leave us with few choices such as pivmecillinam, third generation cephalosporins, azithromycin and carbapenems.

Materials and Methods-

The study was conducted at a tertiary care hospital in New Delhi, India from 1st January 2009 to 31st December 2012. 7377 stool samples were received from patients presenting with signs and symptoms of diarrhea or dysentery. Proper instructions were given regarding collection of stool specimens. In case of delay of more than two hours, samples were transported in Cary Blair medium/ buffered glycerol saline. The samples were subjected to microscopic examination to look for presence of red blood cells, pus cells, cysts, trophozoites, helminthic ova and larvae. Stool specimens were inoculated onto MacConkey's agar, Xylose Lysine Desoxycholate agar, and Bile salt agar directly and after enrichment in selenite F broth and APW (Alkaline peptone water). The plates were incubated for 18-24 hours at 37°C. The organisms were identified on the basis of colony characteristics, Grams staining and biochemical reactions. *Shigella* strains identified were serotyped by the slide agglutination test with polyvalent antisera followed by monovalent antisera. (Denka Seiken Co., Ltd., Tokyo, Japan).

Shigella isolates obtained were subjected to antimicrobial susceptibility testing by the disc diffusion method as per the CLSI guidelines (CLSI, 2011) against ampicillin (10 µg), ampicillin/sulbactam (10 µg+10 µg), gentamicin (10 µg), amikacin (30 µg), doxycycline (30 µg), chloramphenicol (30 µg), cotrimoxazole (1.25 µg+23.75 µg), naldixic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), ceftriaxone (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), piperacillin/tazobactam (100 µg+10 µg), imipenem (10 µg), meropenem (10 µg) and ertapenem (10 µg) (HiMedia, Mumbai, India).

Results-

Over a period of four years, a total of 143 strains of *Shigella* (1.94%) were isolated from 7377 stool samples received. *S. flexneri* (89/143, 62.2%) was the most frequent species isolated, followed by *S. boydii* (36/143, 25.2%), *S. sonnei* (22/143, 15.4%) and *S. dysenteriae* (2/143, 1.4%) (Table 1). Most common *S. flexneri* serotypes that were isolated were types 1, 6 and 2, and among *S. boydii* was type 12. Both *S. dysenteriae* belonged to type 1 (Table 1). 46.2% (66/143) of these stool samples showed presence of red blood cells on microscopic examination, while, 75.5% (108/143) samples showed presence of more than 10 pus cells/HPF.

Shigella strains depicted high levels of resistance to several antimicrobial agents. Highest resistance was seen towards cotrimoxazole (90.9%), nalidixic acid (90.9%) and doxycycline (88.8%). Resistance to fluoroquinolones was also high with more than half of the strains showing resistance to ofloxacin (56.6%) and ciprofloxacin (53.8%). Among the aminoglycosides that were tested, resistance was higher towards gentamicin (10.5%) as compared to amikacin (2.1%). In the beta-lactam class of agents, resistance to ampicillin was 63.6%, while 39.9% strains were resistant to ampicillin/ sulbactam combination. The third generation cephalosporins were also seen to be losing their efficacy, with 18.9% - 20.3% resistance to ceftazidime, ceftriaxone and cefotaxime. All the strains, however, maintained their susceptibility to piperacillin tazobactam combination and the carbapemens (Table 2).

Multi-drug resistance, defined as resistance to three or more antimicrobial agents, was seen in 119 out of 143 (83.2%) *Shigella* strains tested. The most common pattern of resistance was Ampicillin- Ampicillin/Sulbactam- Doxycycline- Chloramphenicol- cotrimoxazole- Nalidixic acid- Ciprfloxacin- Ofloxacin, shown by 15 strains, followed by Amoxycillin- Doxycycline- Nalidixic acid, shown by 12 strains. In total, there were 33 patterns of resistance, with resistance to drugs ranging from 1 to 13 in number. Common resistance patterns are shown in Table 3.

Table 1: Isolated serotypes of Shigella

Species	Serotype	Number of isolates
S. dysenteriae (n=2)	**1**	2
S. flexneri (n= 89)	**1**	23
	2	17
	3	13
	4	15
	6	21
S. boydii (n=30)	**1**	3
	4	3
	5	5
	10	3
	12	7
	13	3
	14	4
	16	2
S. sonnei (n=22)		22
Total		143

Table 2: Antimicrobial resistance of various *Shigella* species isolated:

Antimicrobial Agent	*S. dysenteriae*		*S. flexneri*		*S. boydii*		*S. sonnei*		Total	
	No. (n=2)	%	No. (n=89)	%	No. (n=30)	%	No. (n=22)	%	No. (n=143)	%
Ampicillin	2	100	64	71.9	18	60	7	31.8	91	63.6
Ampicillin + sulbactam	2	100	39	43.8	10	33.3	6	27.2	57	39.9
Gentamicin	1	50	12	13.5	0	0	2	9.1	14	10.5
Amikacin	0	0	3	3.4	0	0	0	0	3	2.1
Doxycycline	2	100	80	89.9	26	86.7	19	86.4	127	88.8
Chloramphenicol	2	100	52	58.4	13	43.3	8	36.4	75	52.4
Cotrimoxazole	2	100	79	88.8	30	100	19	86.4	130	90.9
Nalidixic acid	2	100	82	92.1	24	80	22	100	130	90.9
Ciprofloxacin	2	100	45	50.6	16	53.3	14	63.6	77	53.8
Ofloxacin	2	100	46	51.7	18	60	15	68.2	81	56.6
Ceftriaxone	0	0	16	18.0	7	23.3	6	27.3	29	20.3
Ceftazidime	0	0	15	16.9	6	20	6	27.3	27	18.9
Cefotaxime	0	0	16	18.0	7	23.3	6	27.3	29	20.3
Piperacillin + Tazobactam	0	0	0	0	0	0	0	0	0	0
Imipenem	0	0	0	0	0	0	0	0	0	0
Meropenem	0	0	0	0	0	0	0	0	0	0
Ertapenem	0	0	0	0	0	0	0	0	0	0

Table 3: Common patterns of antimicrobial drug resistance

Number of agents	Resistance pattern	Number of isolates
2	C-NA	5
	CO-D	6
3	CO-D-NA	9
	A-D-NA	12
4	A-CO-D-NA	7
	A-C-CO-D	10
	CIP-D-NA-OF	6
5	CIP-CO-D-NA-OF	11
6	C-CO-CIP-D-NA-OF	5
	A-AS-C-CO-D-NA	6
7	A-C-CIP-CO-D-NA-OF	7
8	A-AS-C-CO-CIP-D-NA-OF	15
9	A-AS-CA-CE-CI-CIP-CO-NA-OF	5
11	A-AS-C-CA-CE-CI-CIP-CO-D-NA-OF	11
12	A-AS-C-CA-CE-CI-CIP-CO-D-G-NA-OF	5

A: Ampicillin, AS: Ampicillin/Sulbactam, G: Gentamicin, D: Doxycycline, C: Chloramphenicol, CO: Cotrimoxazole, NA: Nalidixic acid, CIP: Ciprofloxacin, OF: Ofloxacin, CI: Ceftriaxone, CA: Ceftazidime, CE: Cefotaxime.

Discussion-

The frequency with which various *Shigella* species cause infections vary according to the geographic region. A review by Kotloff et al, 1999 mentioned *S. flexneri* as most common species (60%) in developing world, followed by *S. sonnei* (15%), *S. boydii* (6%) and *S. dysenteriae* (6%), while *S. sonnei* was reported to be the most common (77%) in the developed regions. Thus, India being a developing country is expected to have *S. flexneri* as the most frequent species. This is consistent with our finding, where *S. flexneri* was responsible for 62.2% of the cases.
Antimicrobial agents such as sulphonamides, tetracyclines, ampicillin, cotrimoxazole, nalidixic acid and mecillinam have been used previously, in not very distant past (Bennish ML et al, 1992; Mahmood A, 2001; Varsano I et al, 1991). However, during the past few years, *Shigella* species have demonstrated extraordinary prowess in acquiring resistance to all the classes of agents to which it is exposed. These facts are highlighted in our study.

Before 1990s, ampicillin and cotrimoxazole were considered the drugs of choice for treatment of shigellosis (Salam MA et al, 1991). In the beginning, *S. dysenteriae type* I (Bennish M et al, 1985) and *S. sonnei* (Bratoeva MP et al, 1989) showed their capability to develop resistance. In a large outbreak in West Bengal, *S. dysenteriae* type 1 strains were isolated that showed reduced susceptibility to ampicillin and cotrimoxazole. However, these remained sensitive to nalidixic acid (Pal SC et al, 1984). Later, similar resistance profiles were seen among *S. flexneri* strains isolated in that region in 1987 (Dutta P et al, 1989).

In the early 1990s, increasing resistance to these drugs led to use of nalidixic acid as the drug of choice for treatment of shigellosis in India, Bangladesh and some other countries. However, increasing use of nalidixic acid led to a low level of resistance in *S. dysenteriae* type I isolated from an outbreak in the eastern part of country (Sen D et al, 1988). These strains were also resistant to ampicillin, co-trimoxazole and tetracycline. In the middle of 1990s, the resistance to nalidixic acid developed, and with the exception of *S. sonnei*, all strains were also resistant to ampicillin (Dutta S et al, 1998). Eighteen years later, a similar outbreak of bacillary dysentery with high morbidity and mortality was reported in April 2002 among the labourers of tea garden in the same region. These strains were resistant to ampicillin, cotrimoxazole, nalidixic acid and norfloxacin (Pazhani GP et al, 2004).

In the present study, conducted during 2009-12, *Shigellae* showed high degree of resistance to cotrimoxazole (90.9%), nalidixic acid (90.9%) and ampicillin (63.6%.). High degree of resistance to cotrimoxazole (95% and 94.3%) has been also been reported by Niyogi SK et al, 1994 and Nair GB et al, 2010, respectively. However, resistance to nalidixic acid was seen to rise from 59% (Niyogi SK et al, 1994) to 93.5% (Nair GB et al, 2010) in the two studies over a period of 16 years.

With increase in resistance to these agents, fluoroquinolones were used with increasing frequency. Nalidixic acid was recommended by WHO for treatment of shigellosis until 2004, when it was replaced by ciprofloxacin. It was

found to be highly effective. Pazhani GP et al, from Kolkata, reported no resistance to fluoroquinolones during the year 2001 (Pazhani GP et al, 2005). However, in the subsequent years, they reported progressive rise in the resistance to fluoroquinolone: 11%, 15% and 25% in 2002, 2003 and 2004, respectively. In an another study from the same region published in 2010, Nair GB et al, 2010 reported very high resistance to fluoroquinolones (ciprofloxacin- 90.3%, norfloxacin- 83.1%, and ofloxacin- 81.8%). However, in the present study from North India, we have found slightly lower resistance to ciprofloxacin (53.8%) and ofloxacin (56.6%). Similarly, other studies from North India have also reported lower resistance to fluoroquinolones (Taneja N, 2004; Uppal B et al, 2004). This may be due to regional differences.

Third generation cephalosporins are often used if resistance to the other commonly used agents is met with. However, growing resistance to this group of antibiotics is evident from several studies. In 2010, Nair GB et al. 2010 from West Bengal reported 5.2% resistance to ceftriaxone, while in 2011, Urvashi et al, 2011 reported 12.2% resistance. These have increased further as demonstrated in the present study, reaching 18.9% - 20.3%.

Resistance to antimicrobial agents did not differ in different *Shigella species*. S. dysenteraie strains appeared to be more resistant than other species for several agents, but the sample size of *S. dysenteriae* was too small (n=2) to be significant. However, Pazhani GP et al, found that *S. dysenteriae* type 1 and *S. flexneri* were more resistant than other serotypes (Pazhani GP et al, 2005).

A high degree of multidrug resistance to 3 or more antimicrobial agents in *Shigella* has been documented by several workers (Nair GB et al, 2010; Urvashi et al, 2011). Similar results have been seen in the present study, where 119 of 143 (83.2%) strains were found to be multidrug resistant. An interesting feature noted was that the strains were resistant to third generation cephalosporins were also frequently resistant to several other classes of antimicrobials. This may suggest that resistant strains carry other genes mediating to several other classes such as aminoglycosides and fluorquinolones (Spanu T et al, 2002; Sekowska A et al, 2002).

Our study demonstrates that the *Shigella* strains have developed resistance to not only the drugs that were used earlier, but also to the newer agents like the third generation cephalosporins, which form the backbone of treatment of resistant cases. Now, with reports of resistance to even the carbapenems by production of NDM-1 (Walsh TR et al, 2011), *Shigella*e have proved themselves to be fittest in the race for survival.

Conclusions-

Though *Shigella* seems to be developing resistance rapidly, we can play our part by rationalizing the use of antimicrobial agents. It is essential to strictly adhere to antimicrobial stewardship programmes, which are largely lacking in the developing countries. On the basis of current resistance patterns, it is evident that our choices have narrowed considerably. Thus, the drug of choice for severe cases may have to be changed from current ciprofloxacin to an oral third generation cephalosporin in regions where ciprofloxacin resistance is rampant.

References-

1) World Health Organisation. Health topics, Diarrhoea. (2013a): Available from: http://www.who.int/topics/diarrhoea/en/
2) World Health Organisation. Health topics, Dysentery. (2013b): Available from: http://www.who.int/topics/dysentery/en/
3) The United Nations Children's Fund (UNICEF)/World Health Organization (WHO) Diarrhoea: Why children are still dying and what can be done. (2009): Available from: http://whqlibdoc.who.int/publications/2009/9789241598415_eng.pdf
4) Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ et al. (1999): Global burden of *Shigella* infections: Implications for vaccine development and implementation of control strategies. Bull World Health Organ. 77(8):651-66.
5) Khan A, Huq S, Hossain M, Talukdar K, Malek M, Faruque A. (2005): Presumptive Shigellosis: Clinical and laboratory characteristics of Bangladeshi patients. Scand J Infect Dis. 37(2):96-100.
6) David KV, John SM, Sankarapandian V. (2010) Antibiotic therapy for *Shigella* dysentery. Cochrane Database Syst Rev. 1:CD006784.

7) World Health Organization. (2005): Guidelines for the control of shigellosis, including epidemics due to *Shigella dysenteriae* type 1. Geneva: World Health Organization. 11-3.
8) Clinical and Laboratory Standards Institute. (2011): Performance standards for antimicrobial susceptibility testing; Twenty first Informational supplement. Wayne, PA: Clinical and Laboratory Standard Institute. 31(1):M100-S21.
9) Bennish ML, Salam MA, Hossain MA, Myaux J, Khan EH, Chakraborty J et al. (1992): Antimicrobial resistance of *Shigella* isolates in Bangladesh, 1983-1990: increasing frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, and nalidixic acid. Clin Infect Dis. 14:1055-60
10) Mahmood A. (2001): Nalidixic acid is still the drug of choice for shigellosis in Pakistan. J Pak Med Assoc. 51:101.
11) Varsano I, Elditz-Marcus T, Nussinotich M, Elian I. (1991): Comparative efficacy of ceftriaxone and ampicillin for treatment of severe shigellosis in children. J Pediatr. 118:627-32.
12) Salam MA, Bennish ML. (1991): Antimicrobial therapy for shigellosis. Rev Infect Dis. 13 Suppl 1:S332–S341.
13) Bennish M, Eusof A, Kay B, Wierzba T. (1985): Multiresistant *Shigella* infections in Bangladesh. Lancet. 2:441.
14) Bratoeva MP, John Jr JF. (1989): Dissemination of trimethoprim-resistant clones of *Shigella sonnei* in Bulgaria. J Infect Dis. 159:648–53.
15) Pal SC. Epidemic bacillary dysentery in West Bengal, India, 1984. (1984): Lancet. 1:1462.
16) Dutta P, Dutta D, Bhattacharya SK. (1989): Clinical and bacteriological profiles of shigellosis in Calcutta before and after an epidemic (1984–87). Indian J Med Res. 89:132–7.
17) Sen D, Dutta P, Deb BC, Pal SC. (1988): Nalidixic-acid resistant *Shigella dysenteriae* type 1 in eastern India. Lancet. 2: 911.
18) Dutta S, Mahapatra TS, Dutta P, Mitra U, Dasgupta S. (1998): Serotypes and antimicrobial susceptibility patterns of *Shigella species* isolated from children in Calcutta, India. Eur J Clin Microbiol Infect Dis. 17:298–9.
19) Pazhani GP, Sarkar B, Ramamurthy T, Bhattachrya SK, Takeda Y, Niyogi SK. (2004): Clonal multidrug resistant *Shigella dysenteriae* type 1 strains associated with epidemic and sporadic dysenteries in eastern India. Antimicrobial Agents Chemother. 48:681-4.
20) Niyogi SK, Saha MR, De SP. (1994): Enteropathogens associated with acute diarrheal diseases. Indian J Public Health. 38(2):29-32.
21) Nair GB, Ramamurthy T, Bhattacharya MK, Krishnan T, Ganguly S, Saha DR. (2010): Emerging trends in the etiology of enteric pathogens as evidenced from an active surveillance of hospitalized diarrhoeal patients in Kolkata, India. Gut Pathogens. 2(4). Available from: http://www.gutpathogens.com/content/2/1/4.
22) Pazhani GP, Ramamurthy T, Mitra U, Bhattacharya SK, Niyogi SK. (2005): Species diversity and antimicrobial resistance of *Shigella spp*. isolated between 2001 and 2004 from hospitalized children with diarrhoea in Kolkata (Calcutta), India. Epidemiol Infect. 133:1089–95.
23) Taneja N. (2004): Changing Epidemiology of Shigellosis and Emergence of Ciprofloxacin-Resistant *Shigellae* in India. J Clin Microbiol. 45(2):678-9.
24) Uppal B, Arora VM. (2004): Changing resistance pattern of *Shigella* isolates in a Delhi hospital: An alarming trend. Indian J Med Microbiol. 22(3):199-200.
25) Urvashi, Saxena S, Dutta R. (2011): Antimicrobial resistance pattern of *Shigella* species over five years at a tertiary-care teaching hospital in north India**.** J Health Popul Nutr. 29(3):292-5.
26) Spanu T, Luzzaro F, Perilli M, Amicosante G, Toniolo A, Fadda G. (2002): Occurrence of extended-spectrum beta-lactamases in members of the family Enterobacteriaceae in Italy: implications for resistance to beta-lactams and other antimicrobial drugs. Antimicrob Agents Chemother. 46:196-202.
27) Sekowska A, Janicka G, Klyszejko C, Wojda M, Wroblewski M, Szymankiewicz M. (2002): Resistance of Klebsiella pneumoniae strains producing and not producing ESBL (extended-spectrum beta-lactamase) type enzymes to selected non-beta-lactam antibiotics. Med Sci Monit. 8:BR100-4.
28) Walsh TR, Weeks J, Livermore DM, Toleman MA. (2011): Dissemination of NDM-1 positive bacteria in the New Delhi environment and its implications for human health: an environmental point prevalence study. Lancet Infect Dis. 11(5):355-62.