

Brief Original Articles

Trends in antimicrobial resistance in *Shigella* species in Karachi, Pakistan

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Abstract

Background: Shigellosis is a common cause of morbidity, especially in the very young and old, in developing countries. The disease is treated with antibiotics. Surveillance of antimicrobial resistance trends is essential owing to the global emergence of antimicrobial resistance.

Methodology: The study involved 1,573 isolates of *Shigella* species (1996–2007) that were analyzed for trends in antimicrobial resistance.

Results: The majority of the specimens (1046; 66.5%) were from the pediatric population, and of these 887 (84.8%) were under 5 years of age ($p = 0.001$). *S. flexneri* was the most frequent species (54.5%) isolated. Isolation of *S. sonnei* increased from 15.4 % (1996) to 39% (2007) ($p = 0.001$). Although none of the isolates was found sensitive to all the antibiotics tested, 58% ($n = 907$) were resistant to ampicillin and 85% ($n = 1,338$) were resistant to trimethoprim-sulfamethoxazole (TMP-SMX). Out of a total of 198 (12.6%) nalidixic acid resistant isolates, 6 (3.0%) were also resistant to ofloxacin. Overall 1.7 % of isolates were resistant to ofloxacin, 2.4% to ceftriaxone and 2.3% were resistant to combination of ampicillin, nalidixic acid and TMP-SMX.

Conclusion: Ofloxacin is still an effective drug for treatment of acute shigellosis in Pakistan. Emergence of resistance to ceftriaxone in *Shigella* may have grave implications in treatment of severe shigellosis in very young patients.

Keywords: shigellosis, resistance, ofloxacin, ceftriaxone, Pakistan

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Introduction

Infection by any of the *Shigella* species, namely *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei*, may lead to shigellosis. Global burden of shigellosis is estimated to be approximately 164.7 million cases annually with approximately 163.2 million cases from developing countries [1]. Prompt treatment with appropriate antimicrobial agents not only shortens the duration and severity of the illness but also reduces microbial carriage and thus spread of infection in the community.

Emergence of antimicrobial resistance has complicated the empirical therapy for treatment of shigellosis. Ampicillin was replaced by TMP-SMX and nalidixic acid as preferred drugs owing to emergence of resistance in the early 1970s [2]. Resistance to TMP-SMX made prominence in the 1980s with reports of resistant isolates from many countries [3]. More recently, strains resistant to nalidixic acid and those with high Minimum Inhibitory Concentration (MIC) for fluoroquinolone have been reported [3,4,5], alerting clinicians to probable treatment failure. Reports of ceftriaxone

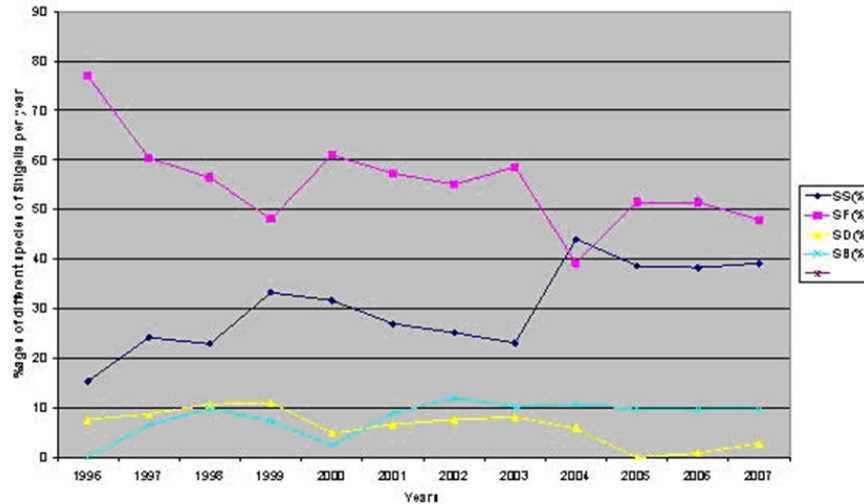
resistance in *Shigella spp.* from various countries owing to ESBL production is another major concern [5,6].

Developing countries such as Pakistan lack infrastructure to monitor anti-microbial resistance at the national level; therefore, guidelines for antimicrobial treatment are limited. Over-the-counter drug availability and self-medication instigates rapid selection and emergence of resistant strains in the community.

This study was conducted to analyze the antibiotic resistance trend in the *Shigella* species at our center to guide clinicians regarding the treatment options of shigellosis in Pakistan. Since Pakistan is one of the most commonly reported regions of imported cases of shigellosis in the developed world, the results of our study would be of international interest. To the best of our knowledge, this is the largest dataset of *Shigella* isolates reported from Pakistan.

Materials and methods

Location and sampling

Figure 1. Frequency of different species of *Shigella* isolates detected at clinical laboratory of Aga Khan University Hospital Karachi (1996-2007).

Aga Khan University Hospital (AKUH) is a 550-bed tertiary care centre located in Karachi, Pakistan. The clinical microbiology laboratory receives samples across the country via its satellite units in more than 50 cities and towns. We analyzed all stool samples submitted for bacterial culture in the laboratory between the years 1996 and 2007. Stool samples were processed according to the standard methodology. Colonies morphologically suggestive of *Shigella* species were identified by conventional biochemical reactions (urea, citrate, triple sugar iron, indole, motility) and slide agglutination tests using antisera from Denka Seiken Co. Ltd, Japan. Non-serotypable isolates were further checked by API 20 E (Bio Murex, France). Antibiotics susceptibility was performed by Kirby Bauer disk diffusion method, against Ampicillin (10ug), Ceftriaxone (30ug), Cotrimoxazole (TMP-SMX) (25ug), Nalidixic acid (30ug) and Ofloxacin (5ug). MIC of selected agents was performed by E-Test (AB Biodisk, Solna, Sweden) according to the manufacturer's recommendations. ESBL production was detected by combined disc method (ceftriaxone alone and ceftriaxone-clavulanic acid). *Escherichia coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 were used as the quality control strains.

Definitions

The pediatric population was defined as children younger than 15 years of age. The antibiotic resistance pattern was defined as follows: Pan sensitive *Shigella* (PSS) *Shigella* isolates sensitive to all the antibiotics tested. The multi-drug resistant *Shigella* species (MDR) was defined as strain

resistant to ampicillin, TMP-SMX, and nalidixic acid. MDR isolates with additional resistance to ofloxacin and /or ceftriaxone were defined as extremely resistant strains (XDR).

Statistical analysis

Analysis was performed using SPSS version 15.0 for Windows (Statistical Package for Social Sciences, Chicago IL, USA). Mean, median, standard deviations (SD) and interquartile ranges (IQR) were calculated for continuous variables. Categorization of independent continuous variables and categorical variables were calculated in a statistically meaningful way.

Descriptive analysis of age, gender, location and other characteristics were computed separately for our study population. The mean and standard deviation was computed for continuous variable by independent t-test. Proportions for categorical variables were calculated and compared by chi-square test.

A detailed stratum specific analysis on ceftriaxone resistance was done among different age groups, different *Shigella* species, and patients' status.

Results

Age and demographics

During the study period (1996-2007) a total of 1,573 samples were positive for *Shigella* species. The majority (1046; 66.5%) were from the pediatric population, and of these 887 (84.8%) were under 5 years of age ($p = 0.001$).

Table 1. Percent resistance of *Shigella* species over period of twelve years isolated at Clinical Laboratory of Aga Khan University Hospital Karachi (1996-2007).

AB	1996	1997	1998	1999	2000	2001	2002	2003	2004	2005	2006	2007	Total	P-value
	n=26	n=91	n=131	n=54	n=41	n=89	n=274	n=355	n=102	n=194	n=115	n=101	n=1573	
SXT	69.2	78	90.1	83.3	80.5	84.3	83.2	92.1	83.3	87.1	73.9	83.2	85.1	<0.001
AMP	73.1	63.7	57.3	59.3	58.5	51.7	60.6	64.5	37.3	58.8	51.3	46.5	57.7	0.002
C	0	0	2.3	11.1	29.3	33.7	34.3	47.3	27.5	37.1	37.4	37.6	31.4	<0.001
NA	0	1.1	6.1	7.4	7.3	2.2	4.7	9.9	19.6	23.0	22.6	41.6	12.6	<0.001
OFX	0	0	0	0	0	1.1	0.4	1.1	2	1	4.3	10.9	1.7	<0.001
CRO	0	0	0	0	0	2.2	1.1	0.8	4	8.2	2	8	2.4	<0.001
PSS	0	0	0	0	0	0	0	0	0	0	0	0	0	-
MDR	0	1	4	7	2	1	3	1	1	3	1	5	2.3	
XDR	0	0	0	0	0	0	0	0	0	0	0	0	0	-

AB: Antibiotic; SXT: septran; AMP: ampicillin; C: chloramphenicol; NA: nalidixic acid; OFX: ofloxacin; CRO Ceftriaxone, PSS: Pan Sensitive *Shigella*; MDR: resistant to three first-line drugs; XDR: MDR + resistance to ofloxacin and/ or ceftriaxone

Monthly distribution

Month-wise distribution of *Shigella* isolation was analyzed during the study period. *Shigella* isolation occurred throughout the year, with significant increases noted in July and August, corresponding with the monsoon season.

Microbiology

S. flexneri was the most frequent species (54.5%) identified in both the adult and pediatric age groups. Isolation of *S. sonnei* progressively increased from 15.4 % (n=4) in 1996 to 33.3% (n = 18) in 1999 and peaked in 2004 to 44.1% (n = 45; p = 0.001). Peak isolation of *S. sonnei* corresponded with a relative fall of *S. flexneri* in that period (Fig.1). Percent isolation of *S. boydii* isolates increased from 0% in 1996 to a peak of 10.1% in 2003 (n = 36). *S. dysenteriae* isolation decreased from 7.7% in 1996 to 0% in 2005 (p = 0.001).

Antibiotic resistance

During the study period resistance to ampicillin and cotrimoxazole remained high with fluctuations. Significant increases in resistance were noted for chloramphenicol, nalidixic acid, and ofloxacin. Ceftriaxone resistance emerged in 2001 and increased to 8% in year 2007. None of the isolates was found to be PSS i.e. sensitive to all the antibiotics tested. A large proportion of isolates was resistant to ampicillin i.e.907 (58%) and TMP-SMX i.e.1338 (85%); 797 (50.7%) of isolates had co-resistance to both drugs (Table 1).

Ampicillin resistance was noticed in all four species, the highest being in *S. flexneri* (n = 763; 84.1%), whereas 4.4% (n = 40) *S. sonnei* isolates were resistant to ampicillin. Resistance to TMP-SMX

was more common among *S. flexneri* isolates than that of *S. sonnei* (54.6% vs. 33.0; p = 0.001).

Resistance to chloramphenicol emerged in 1998 (2.3%; n = 3), and increased to 37.6% (n = 38) in 2007 (p = 0.001). Chloramphenicol resistance was more frequent in *S. flexneri* (86.2%) followed by *S. dysenteriae* and *S. boydii* (6.3% and 4.7% respectively).

Shigella isolates resistant to nalidixic acid were first noted in 1997 with the highest number of resistant isolates 44 (23%) in 2005. Overall resistance to nalidixic acid was common in *S. sonnei* (n = 116; 28.5%) and rare in *S. flexneri* (n = 25; 3.2%; p = 0.001). Resistance to ofloxacin emerged in 2001 and increased to 10.9% in 2007 (n = 11; p = 0.001).

The most alarming finding of this study was detection of resistance to ceftriaxone that emerged in 2001: 65.8% (n = 25) of ceftriaxone resistant isolates were from patients between two and five years of age. Resistance was mainly seen in *S. flexneri* (n = 27; 71.1%) followed by *S. sonnei* (n = 10; 26.3%). The highest number of ceftriaxone resistant isolates was detected in 2005, n = 16 (8.2%). Prior to 2005 ceftriaxone resistance was tested by disc diffusion method; however, from 2005 onwards (due to heightened alert) all resistant isolates were reconfirmed by MIC using E-test (MIC values > 32mg/dl). Ceftriaxone resistant isolates were also resistant to other first line drugs; however, none of these isolates was found resistant to ofloxacin. All the ceftriaxone resistant *Shigella spp.* were ESBL producers.

Thirty-six (2.3%) of the total isolates were resistant to a combination of ampicillin, nalidixic acid and TMP-SMX (MDR). None of the MDR isolates showed additional resistance to ofloxacin and /or ceftriaxone (XDR = 0).

Discussion

Shigellosis is primarily a disease of children and young adults. The majority of shigellosis cases in our population was seen in patients younger than five years. Transmission of *Shigella* commonly occurs by person-to-person contact and through food, water and fomites [7]. Flies play important role in the spread of *Shigella* [8]; therefore, in endemic countries, shigellosis occurs throughout the year peaking during the hot humid and rainy season [9]. Results of our study also reflect this finding with an increase of cases noted in monsoons.

S. flexneri is reported as the most frequent species from Asia [5,10,11]. Although our findings are consistent with this data, our study has revealed an epidemiological shift with progressive increases in *S. sonnei* and *S. boydii* during the study period. Changing epidemiology of shigellosis has been reported from other countries. *S. flexneri* was the most frequent serotype in the United States of America in the early 1960s, but that was replaced by *S. sonnei* during 1964-68 [12]. A recent report from Iran has indicated a shift from *S. flexneri* to *S. sonnei* as the most frequently isolated serotype [13]. Compared with previous reports, Lorenz *et al.* has reported increase in number of *S. boydii* in Bangladesh [11]. Follow-up studies are required to observe the persistence of this finding in our setup.

Antibiotic resistance among *Shigella* species is increasingly being reported from developed as well as developing countries [4,5]. None of the isolates over the period of twelve years in our study was pan sensitive. The proportion of isolates resistant to ampicillin, TMP-SMX, and both increased substantially over the study period. These agents are therefore no longer appropriate for the empirical treatment in our population.

The role of nalidixic acid as an empirical drug for treatment of acute shigellosis in young children is also being compromised in our population, as 12.6% of *Shigella* isolates were resistant to nalidixic acid and of these 3.8% were also resistant to ofloxacin. Our findings are consistent with data from other developing as well as developed countries [3,4, 5]. In addition, nalidixic acid resistant isolates with high MIC for higher generation quinolones have also been reported from the United Kingdom [3].

Overall resistance to ofloxacin in our study isolates was low (1.7%), suggesting that fluoroquinolone is still an effective drug for treatment of acute shigellosis in our population; however, treatment failures due to resistant strains can occur. It

is therefore important that *Shigella* isolates be tested for resistance before treatment is commenced by nalidixic acid and higher generation fluoroquinolones.

Resistance to ceftriaxone has been previously reported from Asian countries [10]. A single case report of ceftriaxone resistant *S. flexneri* was reported from our center in early 2005 [14]; subsequently, the clinical laboratory was put on high alert and all isolates detected (on disc diffusion method) thereafter were confirmed by MICs. Third-generation cephalosporins are mainstays in the treatment of severe forms of diarrhoeal infections, especially in very young children in whom the use of fluoroquinolone is restricted. Resistance to this group of antibiotics is alarming, especially in the backdrop of plasmid mediated extended spectrum beta-lactamase (ESBL) producing *Klebsiella* and other *Enterobacteriaceae* which are increasingly being isolated at our center. All ceftriaxone resistant isolates were ESBL producers and further studies to identify the type of ESBL in these strains are underway at our center.

Our study has some limitations. First, it is a retrospective analysis of laboratory data and findings cannot be generalized to the entire population. The results of our study may be an overestimation of resistance among *Shigella* isolates due to sampling bias. Secondly, most of the antibiotic resistance was detected by disc diffusion method and MIC breakpoints for nalidixic acid and ofloxacin could not be determined. Despite these limitations, the findings of our study have identified changing epidemiology and emerging resistance in our *Shigella* isolates. These findings will certainly guide clinicians (local and international) to the options of treatment of shigellosis in patients within or those traveling from Pakistan.

In conclusion, our study has revealed an epidemiological shift with progressive increase in *S. sonnei* and *S. boydii* in our population. Antibiotic resistance in *Shigella* species at our center has increased at an alarming level. Substantial increase in ampicillin and TMP-SMX resistance precludes use of these agents for empirical treatment in our population. Fluoroquinolone is an option; however, it is important that *Shigella* isolates be tested for resistance before treatment is commenced by nalidixic acid and higher fluoroquinolones. Emergence of resistance to ceftriaxone in *Shigella* strains is a call for concern and an alarm for health

care agencies to implement policies for prudent use of antibiotics.

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Conflict of Interest: None is declared.