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ARTICLE

# Shigellosis in Bay of Bengal Islands, India: clinical and seasonal patterns, surveillance of antibiotic susceptibility patterns, and molecular characterization of multidrug-resistant *Shigella* strains isolated during a 6-year period from 2006 to 2011

**D. Bhattacharya · H. Bhattacharya · R. Thamizhmani · D. S. Sayi · R. Reesu · M. Anwesh · C. Kartick · A. P. Bharadwaj · M. Singhania · A. P. Sugunan · S. Roy**

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**Abstract** This study aims to determine the clinical features and seasonal patterns associated with shigellosis, the antimicrobial resistance frequencies of the isolates obtained during the period 2006–2012 for 22 antibiotics, and the molecular characterization of multidrug-resistant strains isolated from endemic cases of shigellosis in the remote islands of India, with special reference to fluoroquinolone and third-generation cephalosporins resistance. During the period from January 2006 to December 2011, stool samples were obtained and processed to isolate *Shigella* spp. The isolates were evaluated with respect to their antibiotic resistance pattern and various multidrug resistance determinants, including resistance genes, quinolone resistance determinants, and extended-spectrum β-lactamase (ESBL) production. Morbidity for shigellosis was found to be 9.3 % among children in these islands. Cases of shigellosis occurred mainly during the rainy seasons and were found to be higher in the age group 2–5 years. A wide spectrum of resistance was observed among the *Shigella* strains, and more than 50 % of the isolates were multidrug-resistant. The development of multidrug-resistant strains was found to be associated with various drug-resistant genes, multiple mutations in the quinolone resistance-determining region (QRDR), and the presence of plasmid-mediated quinolone-resistant determinants and efflux pump mediators. This report represents the first presentation of the results of long-term surveillance and molecular characterization concerning antimicrobial resistances in clinical *Shigella* strains in these islands. Information gathered as part of the investigations will be instrumental in identifying emerging antimicrobial resistance, for developing treatment guidelines appropriate for that community, and to provide baseline data with which to compare outbreak strains in the future.

D. Bhattacharya · H. Bhattacharya · R. Thamizhmani · D. S. Sayi · R. Reesu · M. Anwesh · C. Kartick · A. P. Sugunan · S. Roy
Regional Medical Research Centre, Indian Council of Medical Research (Department of Health Research, Ministry of Health & Family Welfare, Government of India), Post Bag No. 13, Port Blair, 744101 Andaman & Nicobar Islands, India

D. Bhattacharya · S. Roy (✉)
Regional Medical Research Centre, Indian Council of Medical Research (Department of Health Research, Ministry of Health & Family Welfare, Government of India), NH-4, Nehru Nagar, Belgaum 590010 Karnataka, India
e-mail: roys@icmr.org.in

S. Roy
e-mail: pblicmr@sancharnet.in

A. P. Bharadwaj
Chirayu Child Care Centre, Port Blair, 744101 Andaman & Nicobar Islands, India

M. Singhania
G.B. Pant Hospital, Directorate of Health Services, Port Blair, 744101 Andaman & Nicobar Islands, India

## Introduction

*Shigella* is a major cause of dysentery throughout the world and is responsible for 5–10 % of diarrheal illness in many areas [1]. It has been estimated that 91 million individuals worldwide contract shigellosis each year and, among them, 1.1 million die [2]. About 410,000 (40 %) of these deaths occur among Asian children [3]. Antibiotic therapy reduces the duration of *Shigella dysenteriae* and, therefore, is recommended for the

treatment of moderate to severe dysentery [4]. The appropriate antibiotic treatment of shigellosis depends on identifying resistance patterns [5]. The rapid emergence of resistance warrants the need for the continuous monitoring of susceptibility patterns [6, 7] and the antimicrobial therapy should be governed by periodically updated local antibiotic sensitivity patterns of *Shigella* isolates [4].

Hospital-based bacteriological surveillance initiated in 1994 had identified shigellosis as endemic and a major cause of acute childhood diarrhea in Andaman and Nicobar Islands [8], a centrally administered territory located in the Bay of Bengal of India, with a population of about 380,000. Species and serotypes composition of *Shigella* isolates showed considerable variation over the years [8, 9] and so did their drug resistance patterns [10].

In this study, we have made an attempt to understand the clinical features and seasonal patterns associated with shigellosis, the antimicrobial resistance frequencies of *Shigella* isolates obtained during the period 2006–2011, and characterized the multidrug-resistant strains with special reference to fluoroquinolone and third-generation cephalosporins among the *Shigella* strains isolated from endemic cases of shigellosis in these remote islands of India.

## Methods

### Patients and samples

Pediatric patients (age 0–14 years) attending/admitted to the outpatient ward/ward of various hospitals/primary health centers (G.B. Pant Hospital, Port Blair, INHS Dhanwantari Military Hospital, Port Blair, Chirayu Child Care Centre, Port Blair, and BJR Hospital Car Nicobar) from 1st January 2006 to 31st December 2011 were included in the study. Stool samples were collected and processed for the isolation of *Shigella* spp. Clinical and demographic data were collected via a structured proforma. Samples were collected after obtaining signed consent from the patients/guardians prior to the antibiotic administration. The study was approved by the institutional ethical committee.

### Microbiological examination

The stool samples were processed and the *Shigella* isolates were identified and confirmed following standard techniques [11]. The primary media used for *Shigella* isolation was deoxycholate citrate agar (DCA) and hektoen enteric agar (HEA). The suspected *Shigella* colony after overnight incubation at 37°C was subjected to biochemical characterization and serotyping using group-specific antisera (Denka Seiken Co., Ltd., Tokyo, Japan).

### Antimicrobial sensitivity testing

Antimicrobial sensitivity tests were performed by a disk diffusion method for 22 drugs (Table 1), in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [12]. Control strains of *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 25923 were included in each test. Some of the drugs, such as nitrofurantoin and aminoglycosides, were included even though they are not recommended for the treatment of shigellosis, as resistance to them could be used as a phenotypic characteristic to study the evolution of the pathogen over a period of time.

#### *Detection of ESBL production*

All of the isolates that were resistant to third-generation cephalosporins were tested for the production of extended-spectrum β-lactamase (ESBL) using the combination disk test with ceftazidime–clavulanic acid (CAC, 30/10 μg) and ceftriaxone–clavulanic acid [12, 13].

#### *MICs*

The minimum inhibitory concentrations (MICs) of quinolones (nalidixic acid, ciprofloxacin, and norfloxacin), third-generation cephalosporins (ceftriaxone, ceftazidime, and cefotaxime), and amoxicillin–clavulanate was determined by the Etest (AB Biodisk, Solna, Sweden). The MIC values were interpreted in accordance to the CLSI guidelines [12].

### DNA isolation and PCR amplification

Template DNA was prepared by the heat–chill method [14].

#### *Detection of virulence genes by PCR*

Polymerase chain reaction (PCR)-based detection of virulent genes among the isolated *Shigella* spp. was performed using published primers (Table 2) [15].

#### *Amplification of the QRDRs*

The quinolone resistance-determining regions (QRDRs) of the *gyrA*, *gyrB*, *parC*, and *parE* genes were amplified using published primers (Table 2), as reported previously [16].

#### *Screening of the PMQR determinants*

PCR was performed to screen for the presence of four major groups of *qnr* determinants, *qnrA*, *qnrB*, *qnrC*, *qnrS*, and two

**Table 1** Proportion of *Shigella* strains resistant to antibacterial drugs during the years 2006–2011

| Antimicrobial agents | Percentage resistant to (no.) | | | | |
|---|---|---|---|---|---|
| | *S. flexneri* (*n*=55) | *S. sonnei* (*n*=23) | *S. dysenteriae* (*n*=8) | *S. boydii* (*n*=2) | All *Shigella* isolates |
| Ampicillin | 100.0 (55) | 100.0 (23) | 100.0 (8) | 100.0 (2) | 100.0 (88) |
| Nalidixic acid | 96.4 (53) | 100.0 (23) | 87.5 (7) | 100.0 (2) | 96.6 (85) |
| Tetracycline | 94.5 (52) | 82.6 (19) | 100.0 (8) | 100.0 (2) | 92.0 (81) |
| Norfloxacin | 87.3 (48) | 73.9 (17) | 75.0 (6) | 50.0 (1) | 81.8 (72) |
| Co-trimoxazole | 74.5 (41) | 91.3 (21) | 75.0 (6) | 100.0 (2) | 79.5 (70) |
| Ciprofloxacin | 76.4 (42) | 73.9 (17) | 87.5 (7) | 50.0 (1) | 76.1 (67) |
| Ofloxacin | 74.5 (41) | 65.2 (15) | 75.0 (6) | 50.0 (1) | 71.6 (63) |
| Carbenicillin | 74.5 (41) | 21.7 (5) | 75.0 (6) | 50.0 (1) | 60.2 (53) |
| Azithromycin | 29.1 (16) | 78.3 (18) | 87.5 (7) | 100.0 (2) | 48.8 (43) |
| Amikacin | 29.1 (16) | 52.2 (12) | 62.5 (5) | 50.0 (1) | 38.6 (34) |
| Gatifloxacin | 29.1 (16) | 47.8 (11) | 75.0 (6) | 50.0 (1) | 38.6 (34) |
| Cephalothin (cephalexin) | 36.4 (20) | 34.8 (8) | 62.5 (5) | 50.0 (1) | 38.6 (34) |
| Chloramphenicol | 41.8 (23) | 04.3 (1) | 37.5 (3) | 00.0 (0) | 30.6 (27) |
| Cefuroxime | 25.5 (14) | 08.7 (2) | 50.0 (4) | 50.0 (1) | 23.8 (21) |
| Gentamicin | 21.8 (12) | 13.0 (3) | 25.0 (2) | 100.0 (2) | 21.5 (19) |
| Cefixime | 16.4 (9) | 08.7 (2) | 37.5 (3) | 50.0 (1) | 17.0 (15) |
| Ceftriaxone | 16.4 (9) | 08.7 (2) | 37.5 (3) | 50.0 (1) | 17.0 (15) |
| Cefotaxime | 16.4 (9) | 08.7 (2) | 37.5 (3) | 50.0 (1) | 17.0 (15) |
| Ceftazidime | 16.4 (9) | 08.7 (2) | 37.5 (3) | 50.0 (1) | 17.0 (15) |
| Amoxicillin/clavulanic acid | 16.4 (9) | 08.7 (2) | 37.5 (3) | 50.0 (1) | 17.0 (15) |
| Nitrofurantoin | 07.3 (4) | 04.3 (1) | 37.5 (3) | 50.0 (1) | 10.2 (9) |
| Imipenem | 0 | 0 | 0 | 0 | 0 |

additional plasmid-mediated quinolone resistance (PMQR) genes, *aac(6')-Ib-cr* and *qepA*, using published primers (Table 2) [17].

*Screening of antimicrobial resistance genes*

PCR was performed to detect genes encoding resistance to β-lactams ($bla_{TEM}$, $bla_{OXA-1}$, $bla_{OXA-7}$, $bla_{SHV}$, $bla_{CTX-M3}$, and $bla_{CTX-M14}$), aminoglycosides (*aadB*, *aac3*, *aaaC2*, *aadB*, *aphA1*, and *aphA2*), chloramphenicol (*catA1*), tetracycline [*tet(A)*, *tet(B)*, *tet(C)*, *tet(D)*, *tet(E)*, and *tet(Y)*], trimethoprim (*dfrA1* and *dfrA5*), and sulfonamides (*sulI* and *sulII*) using published primers and conditions (Table 2) [15].

Nucleotide sequencing of the PCR products

PCR products were then subjected to nucleotide sequencing in an automatic sequencer (ABI 3130; Applied Biosystems, Foster City, CA, USA). Contig sequences were edited with SeqScape (Applied Biosystems, Foster City, CA, USA) and compared using the Basic Local Alignment Search Tool (BLAST) of the NCBI database.

Efflux pump assay

Fluoroquinolone-sensitive and -resistant strains of *Shigella* were grown to mid-exponential phase in LB ($OD_{600}$ 0.4) and harvested. Carbonyl cyanide m-chlorophenylhydrazone (CCCP) was added to Müller–Hinton agar (MHA) at a concentration of 20 mg/L. The MHA plate without and with CCCP (20 mg/L) was then used to test the MIC of the fluoroquinolones (ciprofloxacin and norfloxacin) using the Etest (AB Biodisk, Solna, Sweden). Experiments were performed in triplicate after the addition of CCCP to the culture media, as an inhibitor of the proton-motive force, at a final concentration of 100 mM. The potential of CCCP by itself to inhibit the growth of *Shigella* spp. was tested and found to be suitable for the growth of shigellosis at a final concentration of 20 mg/L.

Statistical analysis

The proportions of isolated *Shigella* strains resistant to each of the antibacterial drugs were calculated for each of the *Shigella* spp. separately and were compared for statistical significance by the $\chi^2$ test using Epi Info 7 software (http://www.

**Table 2** Details of the primers, annealing temperature, and amplicon size of the various polymerase chain reaction (PCR) amplifications

| Gene | Sequence (5′–3′) | Annealing temperature (°C) | Amplicon size (bp) |
|---|---|---|---|
| *ipaH* | TGGAAAAACTCAGTGCCTCT<br>CCAGTCCGTAAATTCATTCT | 55 | 423 |
| *ial* | CTGGATGGTATGGTGAGG<br>GGAGGCCAACAATTATTTCC | 55 | 320 |
| *sen* | ATGTGCCTGCTATTATTTAT<br>CATAATAATAAGCGGTCAGC | 55 | 799 |
| *set1A* | TCACGCTACCATCAAAGA<br>TATCCCCCTTTGGTGGTA | 55 | 309 |
| *set1B* | GTGAACCTGCTGCCGATATC<br>ATTAGTGGATAAAAATGACG | 55 | 147 |
| *stx* | ACCCTGTAACGAAGTTTGCG<br>ATCTCATGCGACTACTTGAC | 55 | 140 |
| $bla_{\mathrm{TEM}}$ | GAGTATTCAACATTTTCGT<br>ACCAATGCTTAATCAGTGA | 50 | 857 |
| $bla_{\mathrm{SHV}}$ | TCGCCTGTGTATTATCTCCC<br>CGCAGATAAATCACCACAATG | 50 | 768 |
| $bla_{\mathrm{OXA1}}$ | GCAGCGCCAGTGCATCAAC<br>CCGCATCAAATGCCATAAGTG | 50 | 198 |
| $bla_{\mathrm{OXA7}}$ | AGTTCTCTGCCGAAGCC<br>TCTCAACCCAACCAACCC | 50 | 591 |
| $bla_{\mathrm{CTX\text{-}M3}}$ | AATCACTGCGTCAGTTCAC<br>TTTATCCCCCACAACCCAG | 50 | 701 |
| $bla_{\mathrm{CTX\text{-}M14}}$ | TACCGCAGATAATACGCAGGTG<br>CAGCGTAGGTTCAGTGCGATCC | 50 | 355 |
| *aadB* | TCCAGAACCTTGACCGAAC<br>GCAAGACCTCAACCTTTTCC | 50 | 700 |
| *aaaC2* | CGGAAGGCAATAACGGAG<br>TCGAACAGGTAGCACTGAG | 50 | 740 |
| *aac(3)* | GTGTGCTGCTGGTCCACAGC<br>AGTTGACCCAGGGCTGTCGC | 50 | 627 |
| *aphA1* | ATGGGCTCGCGATAATGTC<br>CTCACCGAGGCAGTTCCAT | 50 | 600 |
| *aphA2* | GAACAAGATGGATTGCACGC<br>GCTCTTCAGCAATATCACGG | 50 | 680 |
| *tet(A)* | GTGAAACCCAACATACCCC<br>GAAGGCAAGCAGGATGTAG | 50 | 888 |
| *tet(B)* | CCTTATCATGCCAGTCTTGC<br>ACTGCCGTTTTTTCGCC | 50 | 774 |
| *tet(C)* | ACTTGGAGCCACTATCGAC<br>CTACAATCCATGCCAACCC | 50 | 881 |
| *tet(D)* | TGGGCAGATGGTCAGATAAG<br>CAGCACACCCTGTAGTTTTC | 50 | 827 |
| *tet(E)* | TTAATGGCAACAGCCAGC<br>TCCATACCCATCCATTCCAC | 50 | 853 |
| *tet(Y)* | ACCGCACTCATTGTTGTC<br>TTCCAAGCAGCAACACAC | 50 | 823 |
| *catI* | AGTTGCTCAATGTACCTATAACC<br>TTGTAATTCATTAAGCATTCTGCC | 50 | 547 |
| *dfrA1* | AAGAATGGAGTTATCGGGAATG<br>GGGTAAAAACTGGCCTAAAATTG | 50 | 391 |
| *dfrA5* | CTGCAAAAGCGAAAAACGG<br>AGCAATAGTTAATGTTTGAGCTAAAG | 50 | 432 |
| *sulI* | TTCGGCATTCTGAATCTCAC<br>ATGATCTAACCCTCGGTCTC | 50 | 822 |

**Table 2** (continued)

| Gene | Sequence (5′–3′) | Annealing temperature (°C) | Amplicon size (bp) |
|---|---|---|---|
| *sulII* | CGGCATCGTCAACATAACC<br>GTGTGCGGATGAAGTCAG | 50 | 722 |
| *gyrA* | TACACCGGTCAACATTGAGG<br>TTAATGATTGCCGCCGTCGG | 64 | 648 |
| *gyrB* | TGAAATGACCCGCCGTAAAGG<br>GCTGTGATAACGCAGTTTGTCCGGG | 64 | 309 |
| *parC* | GTCTGAACTGGGCCTGAATGC<br>AGCAGCTCGGAATATTTCGACAA | 64 | 249 |
| *parE* | ATGCGTGCGGCTAAAAAAGTG<br>TCGTCGCTGTCAGGATCGATAC | 64 | 290 |
| *qnrA* | ATTTCTCACGCCAGGATTTG<br>GATCGGCAAAGGTTAGGTCA | 64 | 516 |
| *qnrB* | GATCGTGAAAGCCAGAAAGG<br>ATGAGCAACGATGCCTGGTA | 64 | 476 |
| *qnrC* | GGGTTGTACATTTATTGAATCG<br>CACCTACCCATTTATTTTCA | 64 | 307 |
| *qnrS* | GCAAGTTCATTGAACAGGGT<br>TCTAAACCGTCGAGTTCGGCG | 64 | 428 |
| *aac(6')-Ib-cr* | TTGCGATGCTCTATGAGTGGCTA<br>CTCGAATGCCTGGCGTGTTT | 55 | 482 |
| *qepA* | AACTGCTTGAGCCCGTAGAT<br>GTCTACGCCATGGACCTCAC | 55 | 596 |

cdc.gov/epiinfo/). A $p$-value of <0.05 was considered to be statistically significant.

## Results

### Patients and isolates

During the period from 1st January 2006 to 31st December 2011, a total of 943 patients were included in the study. Of these 943 patients, 555 (58.9 %) were male and 388 (41.1 %) were female.

Eighty-eight *Shigella* isolates were obtained from these 943 pediatric patients, giving a proportional morbidity for shigellosis of 9.3 % among children in these islands. No deaths due to shigellosis were reported during the study period. Of these 88 *Shigella* isolates, 55 (62.5 %) were *S. flexneri*, 23 (26.1 %) were *S. sonnei*, 8 (9.1 %) were *S. dysenteriae*, and 2 (2.3 %) were *S. boydii*. Throughout the study period, *S. flexneri* was the most prevalent serogroup, except during the year 2009, when *S. sonnei* was isolated from the majority of the shigellosis patients (Fig. 1). Fifty-eight (65.9 %) of the patients positive for *Shigella* spp. were male and the remaining 30 (34.1 %) were female. The difference was statistically significant.

### *Clinical presentation and symptoms*

Various clinical presentations, such as mucous stool, dysentery, vomiting, fever, and severe dehydration, were found to be largely associated with the shigellosis patients than among the non-shigellosis diarrhea patients (Table 3). No significant difference was observed among these clinical presentations between shigellosis and non-shigellosis patients.

### *Age-wise distribution of the patients positive for Shigella isolation*

The distribution of diarrheal cases included in the study by age group showed a large peak among the age group 2–5 years, where 50.4 % (475 of 943) of diarrheal cases occurred. The maximum number of *Shigella* isolates, 72.7 % (64 of 88), was also obtained from this age group. A total of 11 (12.5 %) and 13 (14.7 %) of the *Shigella* isolates were obtained from the patients belonging to the age groups 0–1 and 6–14 years, respectively.

### *Seasonal variation of the Shigella strains*

The distribution of shigellosis cases by month showed a large peak in isolations during May–June of each year. During the years 2009–2011, a small peak in isolation was also observed during the months of September and October. Cases of shigellosis were found to be low during the winter seasons.

### Antibiotic sensitivity among *Shigella* strains

A wide spectrum of antibiotic resistance was observed among the *Shigella* strains obtained during the period

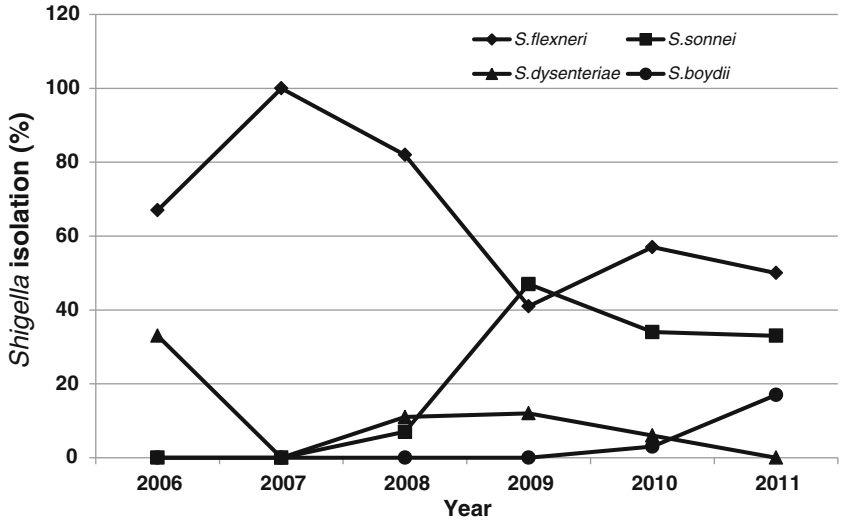


**Fig. 1** Year-wise isolation frequency (%) of *Shigella* serogroups in Andaman and Nicobar Islands (2006–2011)

2006–2011. Forty-four percent of the isolates were resistant to more than ten drugs. All of the *Shigella* strains were resistant to ampicillin. Resistance to commonly used drugs were also observed among the isolated *Shigella* spp., such as nalidixic acid (85, 96.6 %), tetracycline (81, 92 %), norfloxacin (72, 81.8 %), co-trimoxazole (70, 79.5 %), ciprofloxacin (67, 76.1 %), and ofloxacin (63, 71.6 %) (Table 1).

Fluoroquinolone resistance among *Shigella* strains in these islands

During the study period, out of 88 *Shigella* strains, 85 (97 %) were resistant to nalidixic acid, 11 of which were resistant only to nalidixic acid (not resistant to any other drug belonging to the quinolones group). More than 70 % of the isolated *Shigella* spp. were resistant to ciprofloxacin, norfloxacin, and ciprofloxacin (Table 1). The MIC of the isolates ranged from 64 to >256 mg/L for nalidixic acid, 4 to >256 mg/L for ciprofloxacin, and 16 to >256 mg/L for norfloxacin (Table 3).

Third-generation cephalosporins resistance and production of ESBL

During the study period, 15 (17 %) stains of *Shigella* spp. were found to be resistant to all four third-generation cephalosporins tested (cefixime, ceftriaxone, cefotaxime, and ceftazidime) (Table 1). The MICs of the resistant isolates ranged from 32 to >256 mg/L for ceftriaxone, 4 to >256 mg/L for cefotaxime, 4 to >256 mg/L for ceftazidime, and 4 to >256 mg/L for amoxicillin–clavulanic acid. All of the cephalosporin-resistant *Shigella* strains were confirmed to produce ESBL using the combination disk test.

Presence of virulence genes

All of the *Shigella* strains showed the presence of invasive plasmid antigen (*ipaH*). The set genes *set1A* and *set1B* that code for *Shigella* enterotoxin 1 (ShET1) were present in all 44 *S. flexneri 2* isolates, but not in any other serotypes. The *sen* gene coding for *Shigella* enterotoxin 2 (ShET2) was present in 36 (40.9 %) of the 88 *Shigella* strains, which includes 22

**Table 3** Clinical presentation of the pediatric diarrhea patients with and without shigellosis

| Clinical features | No. of patients with shigellosis ($n=88$) | No. of patients without shigellosis ($n=855$) | $p$-Value | Odds ratio (OR) |
|---|---|---|---|---|
| Rice water stool | 0 | 34 (4 %) | 0.1085 | 0.0000 |
| Stool with mucous | 29 (33 %) | 180 (21 %) | 0.0153 | 1.8432 |
| Stool with blood | 7 (8 %) | 77 (9 %) | 0.8941 | 0.8732 |
| Stool with blood and mucous | 7 (8 %) | 68 (8 %) | 0.8364 | 1.0002 |
| Vomiting | 50 (57 %) | 391 (46 %) | 0.0611 | 1.5614 |
| Fever | 57 (65 %) | 301 (35 %) | 0.0000 | 3.3842 |
| Abdominal cramp | 73 (83 %) | 719 (84 %) | 0.9007 | 0.9205 |
| Severe dehydration | 13 (15 %) | 74 (9 %) | 0.0900 | 1.8294 |
| Moderate to low dehydration | 75 (85 %) | 780 (91 %) | 0.5547 | 0.0988 |

(50 %) of the 44 *S. flexneri*, 5 (62.5 %) of 8 *S. dysenteriae*, and 7 (87.5 %) of 8 *S. sonnei*. The *sen* gene was present in 100 % of *S. boydii* and *S. dysenteriae 1* isolates. Nine (10.2 %) of the 88 isolates contained the invasion-associated locus *(ial)* gene. It was present in two (4.5 %) of 44 *S. flexneri* isolates, in all (100 %) of the *S. dysenteriae 1* isolates, in 4 (80 %) of the *S. dysenteriae 2* isolates, and in 1 (100 %) *S. dysenteriae 3* isolate. The Shiga toxin (*stx*) gene was observed in both isolates (100 %) of *S. dysenteriae 1* 1, but not in any other *Shigella* spp.

Detection of antimicrobial resistance genes among the *Shigella* isolates (Table 4)

*Beta-lactams*

All 88 *Shigella* strains were positive for the $bla_{\mathrm{TEM}}$ gene. Of the 88 *Shigella* strains, only 2 (2 %) showed the presence of the $bla_{\mathrm{SHV}}$ gene, of which one isolate was resistant to third-generation cephalosporins. All 15 third-generation cephalosporins-resistant isolates showed the presence of the $bla_{\mathrm{TEM}}$, $bla_{\mathrm{OXA1}}$, and $bla_{\mathrm{CTX\text{-}M3}}$ genes. None of the isolates harbored the $bla_{\mathrm{OXA7}}$ and $bla_{\mathrm{CTX\text{-}M14}}$ genes.

*Aminoglycosides*

Of the 88 *Shigella* strains, 19 isolates were found to be resistant to gentamicin by the disk diffusion method. All 19 (22 %) strains harbored the *aaac2* gene. None of the strains showed the presence of the *aadB*, *aac(3)*, *aphA1*, or *aphA2* genes.

*Tetracycline*

Out of the 88 *Shigella* strains, 81 strains were found to be resistant to tetracycline by the disk diffusion method. All 81 (92 %) resistant isolates harbored the *tetB* gene and 79 (90 %) harbored the *tetA* gene. Among these 79 *Shigella* strains harboring the *tetA* gene, 76 were resistant and three showed intermediate resistance towards tetracycline. None of the strains harbored the *tetC*, *tetD*, *tetE*, or *tetY* genes.

*Phenicols*

A total of 27 strains were found to be resistant to chloramphenicols. A total of 28 (32 %) *Shigella* strains harbored the *catI* gene, including 27 resistant strains and one strain having intermediate resistance towards chloramphenicols.

*Trimethoprim*

Out of 88 *Shigella* strains, 70 were resistant to co-trimoxazole. Seventy-one (81 %) strains harbored the *dfrA1* gene, including 70 resistant strains and one strain having intermediate resistance towards co-trimoxazole. A total of 69 (78 %) *Shigella* strains harbored the *dfrA5* gene, which includes 61 resistant strains and eight strains having intermediate resistance.

*Sulfonamides*

A total of 70 strains were resistant to co-trimoxazole, of which 64 (73 %) harbored the *sulI* gene. However, the *sulII* gene was not detected in any of the strains.

Detection of mutations in the QRDR region (Table 4)

*gyrA*

All of the 11 quinolone (only nalidixic acid)-resistant strains had a single mutation at codon 83 (TCG-TTG), resulting in replacement of serine with leucine (S83L) in *gyrA*. All 74 fluoroquinolone (at least one drug of the group)-resistant strains had double mutations at S83L and at codon position 87 (GAC-AAC/GGC/TAC), resulting in the replacement of D87N (55, 74.3 %)/G (18, 24.3 %)/Y (1, 1.4 %). No mutations were detected in the quinolone-sensitive strains.

*parC*

All of the fluoroquinolone (at least one drug)-resistant strains had a single mutation at codon 80 (AGC-ATC), resulting in the replacement of serine with isoleucine (S80I) in *parC*. Out of the 11 quinolone-resistant strains of *Shigella*, only one had the S80I mutation. No mutations were detected in the quinolone-sensitive strains.

*parE*

Two mutations at codon position 408 (GAC-GGC), resulting in the replacement of aspartic acid with glycine (D408G), and at codon position 458 (TCG-GCG), resulting in the replacement of serine with alanine (S458A), were detected in the *parE* region of the fluoroquinolone-resistant strains of *Shigella*. None of the fluoroquinolone-resistant *Shigella* isolates were found to have both of the mutations simultaneously. Out of the 74 fluoroquinolone-resistant isolates, 36 (48.6 %) *Shigella* strains had D408G and 8 (10.8 %) strains showed S458A. No *parE* mutations were detected in the quinolone-sensitive strains.

No *parC* and *parE* mutations were observed in the 11 *Shigella* isolates which were only nalidixic acid-resistant.

*Prevalence of plasmid-mediated quinolone-resistance determinants*

Among the 85 (out of 88) quinolone- and fluoroquinolone-resistant *Shigella* strains, nine *Shigella* strains were found to

**Table 4** Details of the quinolone resistance-determining region (QRDR) mutations, plasmid-mediated quinolone resistance (PMQR) determinants, efflux pump assay, and prevalence of the antibiotic resistance conferring genes among the isolated *Shigella* spp

| Sl no. | Strain | MIC (mg/L) | | | *gyrA* | | | | *parC* | *parE* | | *qnrB* | *aac(6')-Ib-cr* | Efflux pump assay MIC (mg/L) | | Antibiotic resistance conferring genes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NAL | CIP | NOR | S83L | D87N | D87Y | D87G | S80I | D408G | S458A | | | NOR+CCCP | CIP+CCCP | |
| 1 | *S. dysenteriae 1* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | − | 128 | 256 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 2 | *S. flexneri 2a* | >256 | 2 | 16 | + | − | − | + | + | + | − | − | − | 8 | 0.125 | $bla_{TEM}$, *aaaC2*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 3 | *S. flexneri 2a* | >256 | 128 | 128 | + | + | − | − | + | − | − | − | − | 32 | 64 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, |
| 4 | *S. flexneri 2a* | >256 | 64 | 16 | + | + | − | − | + | + | − | − | − | 12 | 32 | $bla_{TEM}$, *catI*, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 5 | *S. flexneri 2a* | >256 | 32 | 128 | + | − | − | + | + | − | − | − | − | 64 | 4 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 6 | *S. flexneri 2a* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 32 | 32 | $bla_{TEM}$, *aaaC2*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 7 | *S. flexneri 2a* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 64 | 64 | $bla_{TEM}$, *catI*, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 8 | *S. flexneri 4a* | >256 | >256 | 256 | + | + | − | − | + | + | − | − | − | 128 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 9 | *S. dysenteriae 2* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | + | 256 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 10 | *S. flexneri 2a* | >256 | 2 | 32 | + | + | − | − | + | + | − | − | − | 32 | 2 | $bla_{TEM}$, *dhfrV*, *tetB*, *tetA* |
| 11 | *S. flexneri 2a* | >256 | 32 | 256 | + | + | − | − | + | + | − | − | − | 128 | 16 | $bla_{TEM}$, *tetB*, *tetA* |
| 12 | *S. sonnei* | 128 | 0.1 | 2 | + | − | − | − | − | − | − | − | − | 2 | 0.125 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 13 | *S. flexneri 4a* | 64 | 1 | 1 | + | − | − | − | − | − | − | − | − | 1 | 1 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 14 | *S. flexneri 2a* | >256 | 128 | 32 | + | + | − | − | + | + | − | − | − | 12 | 32 | $bla_{TEM}$, *catI*, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 15 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | − | 256 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 16 | *S. flexneri 2a* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 64 | 32 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *catI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 17 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | − | 128 | 128 | $bla_{TEM}$, *catI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 18 | *S. flexneri 2a* | >256 | 128 | >256 | + | + | − | − | + | + | − | − | − | 256 | 32 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 19 | *S. flexneri 2a* | >256 | 128 | >256 | + | + | − | − | + | + | − | − | − | 256 | 32 | $bla_{TEM}$, *tetB*, *tetA* |
| 20 | *S. flexneri 2a* | >256 | 64 | >256 | + | + | − | − | + | + | − | − | − | 256 | 32 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 21 | *S. sonnei* | 128 | 1 | 4 | + | − | − | − | − | − | − | − | − | 4 | 1 | $bla_{TEM}$, *dhfrV* |
| 22 | *S. flexneri 2a* | >256 | 32 | 12 | + | + | − | − | + | + | − | − | − | 12 | 32 | $bla_{TEM}$, *tetB*, *tetA* |
| 23 | *S. flexneri 2a* | >256 | 2 | 32 | + | + | − | − | + | + | − | − | − | 32 | 2 | $bla_{TEM}$, *tetB*, *tetA* |
| 24 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | − | 256 | 256 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 25 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | − | − | − | + | 256 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *catI*, *dhfrV*, *tetB*, *tetA* |
| 26 | *S. dysenteriae 2* | >256 | >256 | 128 | + | + | − | − | + | − | + | − | + | 64 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 27 | *S. flexneri 2a* | >256 | 32 | 128 | + | + | − | − | + | + | − | − | − | 64 | 24 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 28 | *S. flexneri 3b* | >256 | 64 | 64 | + | − | − | + | + | − | − | − | − | 64 | 64 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *tetB*, *tetA* |

**Table 4** (continued)

| Sl no. | Strain | MIC (mg/L) | | | *gyrA* | | | | *parC* | *parE* | | *qnrB* | *aac(6')-Ib-cr* | Efflux pump assay MIC (mg/L) | | Antibiotic resistance conferring genes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NAL | CIP | NOR | S83L | D87N | D87Y | D87G | S80I | D408G | S458A | | | NOR+CCCP | CIP+CCCP | |
| 29 | *S. flexneri 3b* | >256 | 128 | 128 | + | − | − | + | + | − | − | − | − | 64 | 64 | *bla*$_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 30 | *S. flexneri 4a* | >256 | >256 | 64 | + | + | − | − | + | − | + | − | + | 128 | 256 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 31 | *S. flexneri 2a* | 128 | 1 | 4 | + | − | − | − | − | − | − | − | − | 4 | 1 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 32 | *S. dysenteriae 2* | >256 | 4 | 4 | + | + | − | − | + | − | − | − | − | 4 | 4 | *bla*$_{TEM}$, *bla*$_{SHV}$, *tetB*, *tetA* |
| 33 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | − | − | − | − | 256 | 256 | *bla*$_{TEM}$, *aaaC2*, *tetB*, *tetA* |
| 34 | *S. flexneri 2a* | >256 | 64 | 32 | + | + | − | − | + | + | − | − | − | 12 | 24 | *bla*$_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 35 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | − | + | − | − | 256 | 256 | *bla*$_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 36 | *S. flexneri 2a* | >256 | 2 | 2 | + | − | − | − | − | − | − | *qnrB10* | − | 2 | 2 | *bla*$_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 37 | *S. dysenteriae 3* | >256 | >256 | 64 | + | + | − | − | + | − | − | − | + | 16 | 256 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *tetB*, *tetA* |
| 38 | *S. dysenteriae 1* | >256 | 64 | 64 | + | + | − | − | + | − | − | − | − | 32 | 32 | *bla*$_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 39 | *S. flexneri 6* | >256 | 64 | 32 | + | − | + | − | + | − | − | − | − | 32 | 64 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 40 | *S. sonnei* | >256 | 64 | 32 | + | − | − | + | + | − | − | − | − | 16 | 32 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 41 | *S. sonnei* | >256 | 256 | 32 | + | − | − | + | + | − | − | − | − | 24 | 128 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *tetB*, *tetA* |
| 42 | *S. sonnei* | >256 | 64 | 32 | + | − | − | + | + | − | − | − | − | 16 | 32 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 43 | *S. flexneri 2a* | >256 | 2 | 64 | + | − | − | + | + | − | − | − | − | 64 | 2 | *bla*$_{TEM}$, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 44 | *S. sonnei* | 128 | 1 | 2 | + | − | − | − | − | − | − | − | − | 2 | 1 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 45 | *S. sonnei* | >256 | 128 | 32 | + | − | − | + | + | − | − | − | − | 32 | 128 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *tetB*, *tetA* |
| 46 | *S. sonnei* | >256 | 64 | 32 | + | − | − | + | + | − | − | − | − | 32 | 64 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 47 | *S. sonnei* | >256 | 0.1 | 2 | + | − | − | − | − | − | − | − | − | 2 | 0.1 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB* |
| 48 | *S. flexneri 2a* | >256 | 32 | 32 | + | + | − | − | + | + | − | − | − | 32 | 32 | *bla*$_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 49 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | − | + | − | − | 256 | 256 | *bla*$_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 50 | *S. sonnei* | >256 | >256 | 128 | + | + | − | − | + | − | − | − | − | 64 | 256 | *bla*$_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 51 | *S. flexneri 2a* | 128 | 2 | 0.13 | + | − | − | − | + | − | − | − | − | 0.125 | 2 | *bla*$_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *tetB*, |
| 52 | *S. flexneri 1a* | >256 | 1 | 16 | + | − | − | + | + | − | − | − | − | 16 | 1 | *bla*$_{TEM}$, *tetB* |
| 53 | *S. dysenteriae 2* | − | − | − | − | − | − | − | − | − | − | − | − | | | *bla*$_{TEM}$, *tetB* |
| 54 | *S. flexneri 3a* | >256 | 32 | 32 | + | − | − | + | + | − | − | − | − | 32 | 32 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 55 | *S. sonnei* | >256 | 32 | 64 | + | − | − | + | + | − | − | − | − | 64 | 32 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetA* |
| 56 | *S. sonnei* | >256 | 32 | 128 | + | − | − | + | + | − | − | − | − | 64 | 16 | *bla*$_{TEM}$, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 57 | *S. sonnei* | >256 | >256 | >256 | + | + | − | − | + | − | + | *qnrB1* | + | 256 | 256 | *bla*$_{TEM}$, *OXA1*, *CTX-M3*, *bla*$_{SHV}$, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 58 | *S. sonnei* | >256 | >256 | >256 | + | + | − | − | + | − | − | − | − | 256 | 256 | *bla*$_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB* |
| 59 | *S. flexneri 2a* | >256 | 128 | 256 | + | + | − | − | + | + | − | − | − | 128 | 64 | *bla*$_{TEM}$, *dhfrV*, *tetB*, *tetA* |

**Table 4** (continued)

| Sl no. | Strain | MIC (mg/L) | | | *gyrA* | | | | *parC* | *parE* | | *qnrB* | *aac(6')-Ib-cr* | Efflux pump assay MIC (mg/L) | | Antibiotic resistance conferring genes |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | NAL | CIP | NOR | S83L | D87N | D87Y | D87G | S80I | D408G | S458A | | | NOR+CCCP | CIP+CCCP | |
| 60 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | + | − | *qnrB1* | + | 256 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 61 | *S. sonnei* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 64 | 64 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 62 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | − | + | − | − | 256 | 256 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 63 | *S. sonnei* | >256 | >256 | >256 | + | − | − | + | + | − | − | − | − | 256 | 256 | $bla_{TEM}$, *dhfrI*, *sulI*, *tetB*, *tetA* |
| 64 | *S. sonnei* | >256 | 32 | 16 | + | + | − | − | + | + | − | − | − | 16 | 32 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 65 | *S. flexneri 2a* | >256 | 12 | 12 | + | + | − | − | + | + | − | − | − | 12 | 12 | $bla_{TEM}$, *tetB*, *tetA* |
| 66 | *S. flexneri X* | − | − | − | − | − | − | − | − | − | − | − | − | | | $bla_{TEM}$, *aaaC2*, *tetB*, *tetA* |
| 67 | *S. flexneri 2a* | >256 | 12 | 32 | + | + | − | − | + | + | − | − | − | 32 | 12 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 68 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | − | 256 | 256 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 69 | *S. flexneri 2a* | >256 | 2 | 16 | + | + | − | − | + | − | − | − | − | 0.25 | 1 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *tetB*, *tetA* |
| 70 | *S. flexneri 4a* | >256 | 128 | 64 | + | + | − | − | + | + | − | − | − | 64 | 128 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *catI*, *dhfrI*, *dhfrV*, *sulI* |
| 71 | *S. sonnei I* | >256 | 32 | 32 | + | + | − | − | + | − | − | − | − | 32 | 32 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetA* |
| 72 | *S. sonnei I* | >256 | >256 | >256 | + | + | − | − | + | − | + | − | + | 256 | 256 | $bla_{TEM}$, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 73 | *S. dysenteriae 2* | >256 | >256 | >256 | + | + | − | − | + | − | + | − | − | 256 | 256 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 74 | *S. sonnei* | >256 | 128 | 128 | + | − | − | + | + | − | − | − | − | 64 | 32 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 75 | *S. flexneri 2a* | >128 | 16 | 64 | + | + | − | − | − | + | − | − | − | 64 | 16 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 76 | *S. flexneri 2a* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 64 | 32 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 77 | *S. boydii 1* | 128 | 2 | 4 | + | − | − | − | − | − | − | − | − | 4 | 2 | $bla_{TEM}$, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 78 | *S. flexneri 2a* | − | − | − | − | − | − | − | − | − | − | − | − | | | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 79 | *S. sonnei* | >256 | >256 | >256 | + | + | − | − | + | − | − | − | − | 256 | 256 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 80 | *S. flexneri X* | >256 | >256 | 128 | + | − | − | + | + | − | − | − | − | 64 | 256 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 81 | *S. flexneri 2a* | >256 | 64 | 128 | + | − | − | + | + | − | − | − | − | 128 | 64 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 82 | *S. flexneri 2a* | >256 | >256 | >256 | + | + | − | − | + | + | − | − | − | 256 | 256 | $bla_{TEM}$, *catI*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 83 | *S. flexneri 2a* | >256 | 1 | 16 | + | + | − | − | + | − | − | − | − | 16 | 1 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 84 | *S. sonnei* | 256 | 0.1 | 4 | + | − | − | − | − | − | − | − | − | 4 | 0.125 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *tetB*, *tetA* |
| 85 | *S. flexneri 2a* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 64 | 64 | $bla_{TEM}$, *dhfrV* |
| 86 | *S. sonnei* | 128 | 0 | 0.03 | + | − | − | − | − | − | − | − | − | 0.032 | 0.032 | $bla_{TEM}$, *dhfrI*, *dhfrV*, *tetB*, *tetA* |
| 87 | *S. boydii 1* | >256 | 128 | 128 | + | + | − | − | + | + | − | − | − | 32 | 64 | $bla_{TEM}$, *OXA1*, *CTX-M3*, *aaaC2*, *dhfrI*, *dhfrV*, *sulI*, *tetB*, *tetA* |
| 88 | *S. flexneri 2a* | 128 | 64 | 128 | + | + | − | − | + | + | − | − | − | 64 | 24 | $bla_{TEM}$, *dhfrI*, *tetB* |

harbor the PMQR determinants, yielding a prevalence of 10.6 % for PMQR genes among *Shigella* spp. (Table 4). Out of 85 isolates, 8 (9.4 %) strains showed the presence of *aac(6')-Ib-cr* and 3 (3.5 %) strains harbored the *qnrB* gene, with 2 (2.4 %) of these strains showing the presence of both. A total of 6 (7 %) strains harbored only *aac(6')-Ib-cr*, while only 1 (1.2 %) harbored only the *qnrB* gene. The strain harboring only the *qnrB* gene was resistant only to nalidixic acid. None of the strains were positive for the *qnrA*, *qnrC*, *qnrS*, or *qepA* genes.

Two strains harbored the *qnrB1* variant, whereas one harbored the *qnrB10* variant.

All of the *Shigella* strains harboring the *aac(6')-Ib-cr* gene had uniform double mutations in *gyrA* (S83L and D87N) and a single mutation in *parC* (S80I). Of the eight strains harboring the *aac(6')-Ib-cr* gene, six had additional mutations in the *parE* gene (D408G in two and S458A in four strains, respectively).

Two strains harboring both the *aac(6')-Ib-cr* and *qnrB* genes had uniform mutations in the *gyrA*, *parC*, and *parE* genes.

### *Role of efflux pump in the development of fluoroquinolone resistance*

Most of the fluoroquinolone-resistant *Shigella* strains, irrespective of the serogroup/serotype, strongly exhibited fluoroquinolone efflux. Out of the 85 fluoroquinolone-resistant *Shigella* isolates tested, 53 (62.4 %) isolates exhibited the efflux pump mechanism, i.e., the MIC of the drug with CCCP was lower than that without CCCP (Table 4). The MIC of norfloxacin and ciprofloxacin was found to be two- to four-fold lower in the resistant strains after the addition of the efflux pump inhibitor, CCCP. The median of the MICs for fluoroquinolones (norfloxacin and ciprofloxacin) in combination with CCCP was found to be lower than that of the MIC of the drug when tested alone (Fig. 2).

## Discussion

The present study, covering the years 2006–2011, demonstrates a proportional morbidity for shigellosis of 9.3 % among children suffering from gastroenteritis in these islands, which is slightly higher than that reported elsewhere and for other Asian countries [18, 19]. Similar to other studies on shigellosis [19], in our study, the incidence of shigellosis was found to be higher among children in the age group<5 years of age.

As other investigators observed in other developing countries [19, 20], *S. flexneri* was the predominant species, with a mean prevalence of 63 %.

Changing patterns of antimicrobial susceptibilities among *Shigella* isolates pose major difficulties in selecting an appropriate drug for the treatment of shigellosis [21]. Over the past few decades, *Shigella* spp. has become resistant to most of the widely used antimicrobials [10, 22].

Multiply resistant strains of *Shigella* spp. have occurred in different geographical regions, viz. Europe [23], Africa [24], Asia [25], and South America [18].

Our results showed the high prevalence of resistance to tetracycline and ampicillin in *Shigella* spp., despite the facts that ampicillin has not been used during the past few years in these islands for the treatment of suspected cases of shigellosis and tetracycline is not used in children [26].

Post emergence of resistance to nalidixic acid, other fluoroquinolones such as ciprofloxacin, norfloxacin, ofloxacin, and third-generation cephalosporins became the primary choice for antibacterial therapy to treat pediatric diarrhea patients in these islands. The present study shows that *Shigella* strains are rapidly acquiring resistance to these drugs as well. All of the cephalosporin-resistant *Shigella* strains were found to produce ESBL. Third-generation cephalosporins resistance among *Shigella* spp. was reported for the first time from France [27]. Since then, many cephalosporin-resistant strains of *Shigella* spp. have been reported from developing countries in Asia [13].

In our study, all the *Shigella* isolates showed the presence of invasive plasmid antigen (*ipaH*). The set genes *set1A* and *set1B* that code for *Shigella* enterotoxin 1 (ShET1) were present in all 44 *S. flexneri 2* isolates, but not in any other serotypes. ShET1 induces the time- and dose-dependent intestinal secretion responsible for the watery phase of *S. flexneri* infections [28]. It is found almost exclusively in *S. flexneri* serotype 2 [29]. This gene has the potential of being used as a marker for the identification of *S. flexneri 2* serotypes.

Ampicillin resistance in *Shigella* isolates described in this study was largely associated with TEM β-lactamase genes, which supports other reports indicating that TEM β-lactamase genes (i.e., *TEM-1* β-lactamase gene) are the most prevalent in ampicillin-resistant Enterobacteriaceae [30]. In accordance to the present study, the predominance of $bla_{\text{OXA-1}}$ in *Shigella* spp. has been reported in many countries [31]. Similar to the reports from Mexico and Brazil [32, 33], we found a high frequency of *tetB* followed by *tetA* among *Shigella* strains. In Gram-negative bacteria, *tetA* and *tetB* efflux genes are widely distributed and normally associated with plasmids, of which most are conjugative [34]. In common with some South American *Shigella* strains [33], the presence of the chloramphenicol resistance gene *catA1*, which encodes chloramphenicol O-acetyltransferase and is responsible for most of the plasmid-mediated resistance to chloramphenicol, was also observed among all chloramphenicol-resistant *Shigella* strains in these islands. The most common mechanism of TMP resistance in Enterobacteriaceae, including *Shigella*, is the acquisition of an additional, plasmid-encoded, variant DHFR enzyme. The most common of these is known as DHFR I, which spreads rapidly on the transposon Tn7, which is

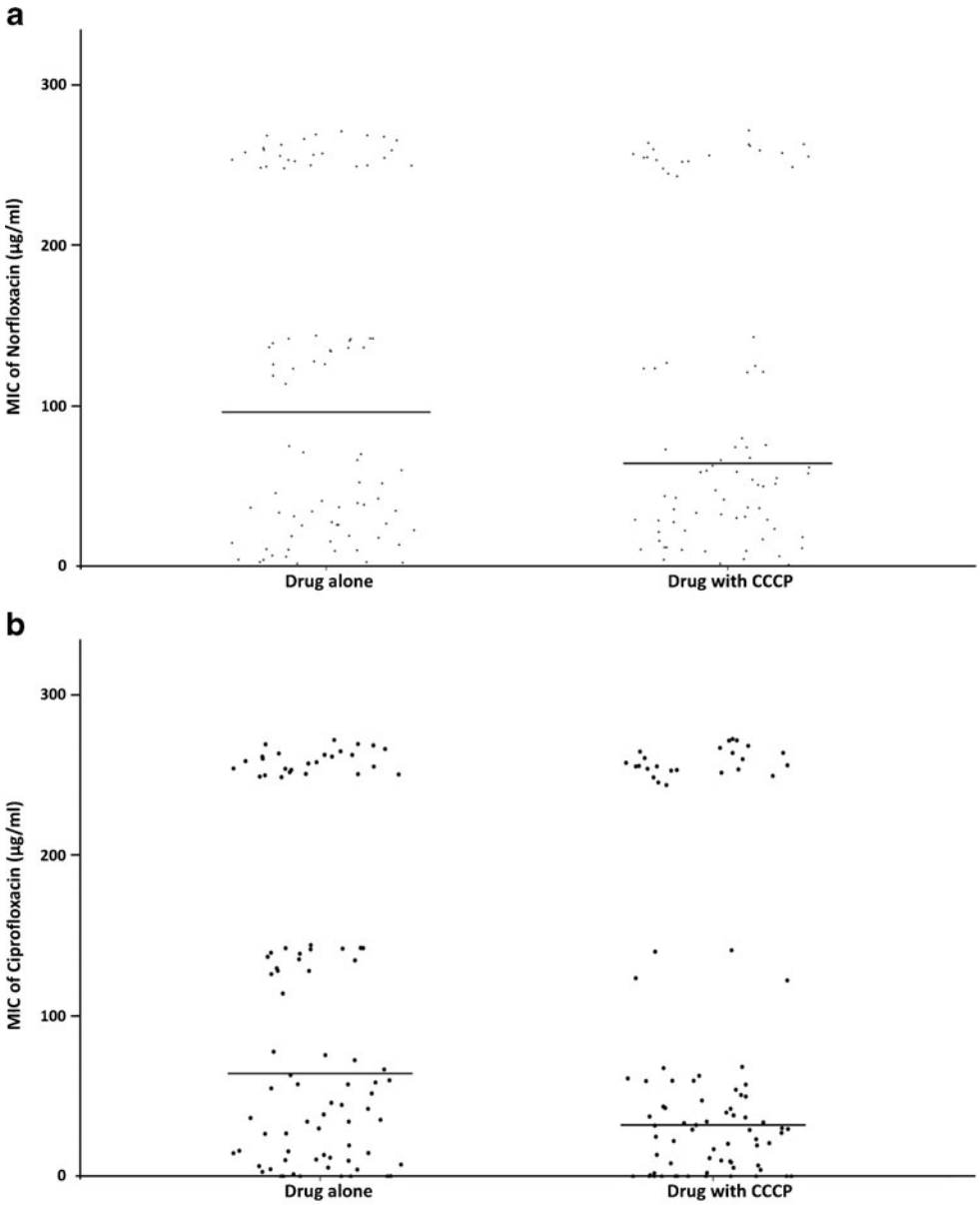


**Fig. 2** Median minimum inhibitory concentrations (MICs) (mg/L) for (**a**) norfloxacin and (**b**) ciprofloxacin alone and in combination with carbonyl cyanide m-chlorophenylhydrazone (CCCP)

promiscuous in nature and thought to have contributed to the rapid dissemination of TMP resistance determinants [33].

Antibiotic resistance can arise in the absence of selective pressures where antibiotic resistance genes are linked on a mobile genetic element. Furthermore, stopping treatment, and the consequent removal of selective pressure, does not necessarily lead to the loss of resistance [35]. Resistance to a range of antimicrobials can, thus, be selected for by administrating one, or a subset, of antimicrobials [36]. However, due to the promiscuous nature of the Shigellae, it is likely that resistance genes are transferred regularly to and from other enteric bacteria and maintained by selective pressure.

The present study shows that quinolone and fluoroquinolone resistance is linked mainly to mutations located in the QRDRs of DNA gyrase (GyrA and GyrB) and topoisomerase IV (ParC and ParE) [37, 38].

As described previously for Enterobacteriaceae [39, 40], the present study showed that nalidixic acid resistance is related mainly to the presence of a single amino acid substitution at either position 83 or position 87 of GyrA, while resistance to ciprofloxacin is related to the presence of at least one additional substitution in GyrA or ParC [39].

In the present study, the prevalence of PMQR genes in *Shigella* spp. was found to be similar to other reports on PMQR prevalence [17]. The current study indicates the presence of these PMQRs as a stepwise phenomenon following the multiple mutations in the QRDR, which, perhaps, play an important role in increasing the resistance towards fluoroquinolones

among *Shigella* isolates in these Islands. Despite the fact that the presently known genes for PMQR are rare [14], our study revealed the presence of *qnrB* in a few *Shigella* isolates from the Andaman Islands. Our study, perhaps for the first time in India, also establishes the presence of the *aac(6')-Ib-cr* gene in *S. dysenteriae* and *S. sonnei*, in addition to *S. flexneri*. These factors, together with the increasing use of fluoroquinolones, created the opportunity for the emergence of highly quinolone-resistant clinical isolates associated with multidrug resistance.

Our result in accordance with earlier studies [14, 41] demonstrated the two- to four-fold decrease in the MIC of fluoroquinolones (CIP and NOR) in more than 50 % of the strains after the addition of uncoupler CCCP, suggesting endogenous energy-dependent efflux. The results clearly suggest that efflux pumps are one of the factors responsible for the development of resistance. Previous studies have shown the role of energy-dependant efflux in the development of resistance of clinical isolates to four structurally unrelated antibiotics, β-lactam, TET, MTZ, and CIP [42].

This report represents the first presentation of the results of long-term surveillance and molecular characterization concerning antimicrobial resistances in clinical *Shigella* strains conducted in these Islands. This study confirms findings from other parts of the world that point to a continued emergence of multidrug-resistant strains of enteric pathogens in the face of widespread antimicrobial use. The emergence of multidrug-resistant *Shigella* isolates strengthens the need for a continuous surveillance system in these remote Islands. The development of a vaccine that is protective against shigellosis caused by multidrug-resistant isolates is a highly desirable public health goal, but the development of such a vaccine is complicated by the variation in species and serogroups between sites, years, and age groups. Information gathered as part of these investigations will be instrumental in identifying emerging antimicrobial resistance, for developing treatment guidelines appropriate for that community, and to provide baseline data with which to compare outbreak strains in the future.

## Nucleotide sequence accession numbers

The sequences in this study have been deposited in the GenBank database under accession numbers HM068906–HM068910, HQ166944–HQ166949, HQ123622–HQ123624, HQ203196–HQ203209, JQ070959–JQ070963, JN972433–JN972437, and HQ246165–HQ246194.

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**Transparency declaration** None to declare.

**Conflict of interest** The authors declare that they have no conflict of interest.

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