

Antimicrobial resistance in *Shigella* - rapid increase & widening of spectrum in Andaman Islands, India

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Background & objectives: Shigellosis is known to be a major cause of acute childhood diarrhoea in Andaman & Nicobar Islands, India. Rapid emergence of antibiotic resistance warrants continuous monitoring of sensitivity pattern of bacterial isolates. We report here the salient findings of an ongoing study on shigellosis in Andaman Islands, India, with regards to change in drug resistance pattern during the past one decade.

Method: During 2006-2009, stools samples from 412 paediatric diarrhoea patients were collected and processed for isolation and identification of *Shigella* spp. Susceptibility to 22 antimicrobial drugs was tested and MICs were determined for 3rd generation cephalosporins, quinolones, amoxicillin-clavulanic acid combination and gentamicin. Drug susceptibility pattern of these isolates were compared with that of 33 isolates obtained during 2000-2002.

Results: *Shigella* isolates were recovered from 50 of 412 stool samples processed. Resistance to ampicillin, nalidixic acid, tetracycline and ciprofloxacin was observed in 100, 96, 94 and 82 per cent of the isolates, respectively. The frequency of resistance to these drugs was significantly ($P<0.001$) higher than that observed during 2000-2002. Resistance to seven drugs was observed in 2000-2002, whereas resistance to 21 drugs was seen during 2006-2009. The number of drug resistance pattern increased from 13 in 2000-2002 to 43 in 2006-2009. Resistance to newer generation fluoroquinolones, 3rd generation cephalosporins and augmentin, which was not observed during 2000-2002, appeared during 2006-2009.

Interpretation & conclusions: The frequency of resistance among *Shigella* isolates has increased substantially between 2000-2002 and 2006-2009 and the spectrum of resistance has widened. At present, the option for antimicrobial therapy in shigellosis in Andaman is limited to a small number of drugs. Continuous local monitoring of resistance patterns is necessary for the appropriate selection of empirical antimicrobial therapy.

Key words Antibiotic - diarrhoea - frequency - paediatric - resistance - *Shigella* - widening spectrum

Shigella is a major cause of dysentery throughout the world and is responsible for 5-10 per cent of diarrhoeal illness in many areas¹. It has been estimated that 91 million individuals worldwide contract shigellosis each year and among them 1.1 million die². About, 4,10,000 (40%) of these deaths occur among Asian children³. Antibiotic therapy reduces the duration of *Shigella dysenteriae* infection and, therefore, is recommended, for the treatment of moderate to severe dysentery⁴. Appropriate antibiotic treatment of shigellosis depends on identifying resistance patterns⁵. Rapid emergence of resistance warrants the need for continuous monitoring of sensitivity patterns^{6,7} and the choice of antibiotic should be governed by periodically updated local antibiotic sensitivity patterns of *Shigella* isolates⁴.

Hospital based bacteriological surveillance has identified shigellosis as endemic and a major cause of acute childhood diarrhoea in Andaman and Nicobar Islands^{8,9}. As in the case of most other developing countries, *S. flexneri 2a* was the commonest isolate. However, species and serotypes composition of *Shigella* isolates showed considerable variation over the years^{8,10} and so did their drug resistance patterns. A study on microbiological, clinical and epidemiological aspects of childhood diarrhoea among the population of Andaman and Nicobar Islands was initiated by Regional Medical Research Centre (ICMR), Port Blair in these Islands a few years back. The objectives of the study were to estimate the proportional morbidity ratio of childhood diarrhoea due to infection with different enteric pathogen, assess the emergence of drug resistance among bacterial enteric pathogens, describe the epidemiology of childhood diarrhoea in the islands and to assess the distribution of toxigenic genes among enteric pathogens and the their association with clinical presentation, severity and outcome of disease. In this report, we describe the drug resistance pattern of the isolates of *Shigellae* obtained as part of the study and a comparison with those obtained earlier.

Material & Methods

Patients and specimens: Diarrhoea patients in the age group of 6 months - 14 yr admitted to the wards of G.B. Pant Hospital and Primary/Community Health Centers present throughout the Andaman & Nirobar Islands from January 2006 to December 2009, were included in the study. Attempt was made to include all available in-patients in the above age group with diarrhoea without adopting any sampling procedure. Stool samples were

collected in stool vials (Hi-Media, Mumbai) prior to the administration of antimicrobials. The samples were immediately brought to the Regional Medical Research Centre (RMRC), Port Blair, laboratory maintaining 4°C for processing. Written consent was taken from the patient/guardian prior to collection of samples. The study was performed at RMRC, Port Blair and the study protocol was approved by the ethics committee of the Centre.

Microbiological examination: The stools samples were processed and the *Shigella* isolates were identified and confirmed following standard techniques¹¹. The primary culture medium for *Shigella* isolation was deoxycholate citrate agar (DCA) (Hi-Media, Mumbai, India) and Hektoen Enteric Agar (HEA) (Hi-Media, Mumbai, India). The isolation of *Shigella* was followed by biochemical characterization and serotyping using group specific antisera (Denka Seiken, Japan). Antibiotic susceptibility testing was carried out using the disc diffusion method, according to Clinical and Laboratory Standards Institute (CLSI) guidelines¹¹ using antibiotic discs (Hi-Media, India) which included ampicillin (AMP, 10 µg), tetracycline (TET, 30 µg), cotrimoxazole (CoT, 20 µg), nalidixic acid (NAL, 30 µg), ciprofloxacin (CIP, 30 µg), gentamicin (GEN, 10 µg), norfloxacin (NOR, 10 µg), nitrofurantoin (NIT, 300 µg), ofloxacin (OFX, 5 µg), gatifloxacin (GAT, 5 µg), amikacin (AMK, 30 µg), azithromycin (AZM, 30 µg), imipenem (IMP, 30 µg), chloramphenicol (CHL, 30 µg), carbenicillin (CAR, 100 µg), cefixime (CFM, 30 µg), cefuroxime (CXM, 5 µg), cephalothin (CEF, 30 µg), ceftriaxone (CRO, 30 µg), cefotaxime (CTX, 30 µg), ceftazidime (CAZ, 30 µg) and augmentin/amoxicillin-clavulanic acid combination (AMC, 30 µg). Control strains *Staphylococcus aureus* ATCC 25923 and *Escherichia coli* ATCC 25922 were included in each test. The minimum inhibitory concentrations (MICs) for cephalosporins (ceftriaxone, ceftazidime and cefotaxime), quinolones (nalidixic acid, ciprofloxacin, ofloxacin and gatifloxacin), augmentin and gentamicin were determined by E-test (AB Biodisk, Sweden). Extended spectrum β-lactamase (ESBL) production was detected using the double-disk synergy method with ceftazidime-clavulanic acid (CAC, 30/10 µg) and ceftriaxone-clavulanic acid (CAC, 30/10 µg).

Some of the drugs such as nitrofurantoin and aminoglycosides were included though these are not recommended for treatment of shigellosis, resistance to these could be used as a phenotypic characteristic to study the evolution of the pathogen over a period of time.

Statistical analysis: The proportions of isolated *Shigella* isolates resistant to each of the antibacterial drugs for 2006-2009 were calculated, and compared with those of 2000-2002, and statistical significance was tested by χ^2 test, or Fisher's exact test when the expected number in any cell was less than five.

Results

Species distribution: Of the 412 stool samples processed, 50 (12.1%) yielded *Shigella* isolates. Among these, 34 (68%) were *S. flexneri*, 10 (20%) *S. sonnei* and 6 (12%) were *S. dysenteriae*. No *S. boydii* was isolated during the study period. In 2006, 2007 and 2008 *S. flexneri* was the dominant strain isolated whereas in 2009 almost equal numbers of *S. flexneri* (7) and *S. sonnei* (8) were isolated.

Antimicrobial resistance: All the 50 *Shigella* isolates obtained during the study period were resistant to ampicillin, 96 per cent to nalidixic acid (MIC, 0.5 to

>256 $\mu\text{g/l}$), 94 per cent to tetracycline, 82 per cent to ciprofloxacin (MIC, 1 to >256 $\mu\text{g/l}$), 80 per cent to ofloxacin (MIC, 1 to >256 $\mu\text{g/l}$) and 70 per cent to norfloxacin (MIC, 0.5 to >256 $\mu\text{g/l}$) (Table). About 40 per cent of the isolates were also resistant to fourth generation fluoroquinolone *viz.*, gatifloxacin (MIC, 2 to 8 $\mu\text{g/l}$). A significant proportion of the isolates were resistant to the two aminoglycosides used in the study *viz.* amikacin (48%) and gentamicin (26%, MIC 0.5 to >256 $\mu\text{g/l}$). Resistance to third generation cephalosporins also appeared to be emerging as 12 per cent showed resistance to the three third generation cephalosporins used *viz.* ceftriaxone (MIC, 30 to >256 $\mu\text{g/l}$), cefotaxime (MIC 5 to >256 $\mu\text{g/l}$) and ceftazidime (MIC 5 to >256 $\mu\text{g/l}$). Some isolates were also resistant to augmentin (12%, MIC 5 to >256 $\mu\text{g/l}$). All the isolates were sensitive to imipenem. Resistance to fluoroquinolones and aminoglycosides was more common among *S. dysenteriae* as compared to *S. flexneri*, but the number of *S. dysenteriae* isolates was small for these differences to be statistically significant. Only five isolates were confirmed to produce ESBL due to an increase of 5 mm in zone diameter around ceftazidime-clavulanic acid and ceftriaxone-clavulanic acid disc compared to the zone around the ceftazidime and ceftriaxone disc respectively¹².

The 50 isolates obtained during the study period (2006-2009) showed 43 drug resistance patterns, all of which involved three or more drugs. Among these drug resistance patterns, one (2.3%) was resistance to three drugs, seven (16.3%) to 4-5 drugs, nine (20.9%) to 6-7 drugs and 13 (30.2%) each to 8-9 drugs and 10 or more drugs. Forty nine (98%) out of the 50 isolates were resistant to four or more drugs.

Thirty three isolates obtained during the period 2000-2002 were retested for the new drugs which were not used at that time. Resistance to 7 of the 22 antibacterial drugs was observed in one or more of the 33 isolates obtained in 2000-2002 whereas resistance to 21 of the 22 drugs was observed during the present period. In 2000-2002, ampicillin and co-trimoxazole were the only two drugs against which more than 50 per cent of the isolates showed resistance while in 2006-2009 there were nine drugs, including norfloxacin, ciprofloxacin, carbenicillin and azithromycin, to which more than 50 per cent of the isolates were resistant. The proportions of resistant isolates showed an increase from 2000-2002 to 2006-2009 in the case of 21 of the 22 antibiotics tested, the only exception being imipenem, resistance to which was observed neither

Table. Proportions of *Shigella* isolates resistant to various antibacterial drugs during 2000-2002 and 2006-09

Antimicrobial agents	No. of isolate resistant to (%)		P (χ^2)
	2000-2002 n = 33	2006-2009 n = 50	
Ampicillin	24 (72.7)	50 (100.0)	<0.001*
Nalidixic acid	09 (27.3)	48 (96.0)	<0.001
Tetracycline	03 (9.1)	47 (94.0)	<0.001
Ciprofloxacin	0 (0.0)	41 (82.0)	<0.001
Ofloxacin	0 (0.0)	40 (80.0)	<0.001
Co-trimoxazole	19 (57.6)	40 (80.0)	0.027
Norfloxacin	03 (9.1)	35 (70.0)	<0.001
Carbenicillin	0 (0.0)	34 (68.0)	<0.001
Azithromycin	0 (0.0)	25 (50.0)	<0.001
Amikacin	0 (0.0)	24 (48.0)	<0.001
Chloramphenicol	09 (27.3)	22 (44.0)	0.123
Gatifloxacin	0 (0.0)	20 (40.0)	<0.001
Gentamicin	0 (0.0)	13 (26.0)	0.001
Cephalexin	0 (0.0)	11 (22.0)	0.002*
Cefuroxime	0 (0.0)	11 (22.0)	0.002*
Cefixime	0 (0.0)	07 (14.0)	0.038*
Nitrofurantoin	01 (3.0)	07 (14.0)	0.137*
Ceftriaxone	0 (0.0)	06 (12.0)	0.076*
Cefotaxime	0 (0.0)	06 (12.0)	0.076*
Ceftazidime	0 (0.0)	06 (12.0)	0.076*
Augmentin	0 (0.0)	06 (12.0)	0.076*
Imipenem	0 (0.0)	0 (0.0)	

*Expected cell frequency less than 5, therefore, P based on Fisher's exact test

in 2000-2002 nor in 2006-2009. This increase in the proportions of resistant isolates between the two study periods was significant ($P < 0.05$) in the case of 15 of the 21 drugs against which resistance was observed. The most noticeable increase in the resistance was against nalidixic acid (27.2 to 96%), ampicillin (72.7 to 100%) and co-trimoxazole (57.6 to 80%). During the present study period, more than 90 per cent isolates were resistant to nalidixic acid, ampicillin, tetracycline and 80-90 per cent of these to the other commonly used drugs such as ciprofloxacin, ofloxacin and co-trimoxazole¹³.

In 2002, the number of drug resistance patterns observed was only 13 whereas during the present study 43 patterns were observed indicating a widening of the spectrum of drug resistance pattern during the intervening period. In 2002, only 21 per cent of the isolates were resistant to more than three drugs and none was resistant to more than five drugs whereas during 2006-2009, 41 of the 50 isolates (82%) were resistant to more than five drugs. The median number of drugs the isolates were resistant was 2 in 2002 and 9 during 2006-2009 (Fig.).

Discussion

Changing patterns of antimicrobial susceptibilities among *Shigella* isolates pose major difficulties in selecting an appropriate drug for the treatment of shigellosis^{14,15}. The present study demonstrated the increasing spectrum of antimicrobial resistance of *Shigella* isolates during the past one decade in these islands. There has been a decline in cases of acute childhood diarrhoea during the two years that followed the Great Asian Tsunami of December 2004¹⁶. However, during 2008-2009 there was an upsurge in the number of cases and the antimicrobial resistance spectra of *Shigella* isolates were much wider than those observed earlier.

Resistance to antimicrobial agents used to treat shigellosis in young children, namely ampicillin, was observed in 1990s in the islands^{8,9}. This has been increasing over the years and at present all the isolated *Shigellae* are resistant to this drug. Presence of resistance to tetracycline showed a ten-fold increase from 9 to 94 per cent during this decade. Nalidixic

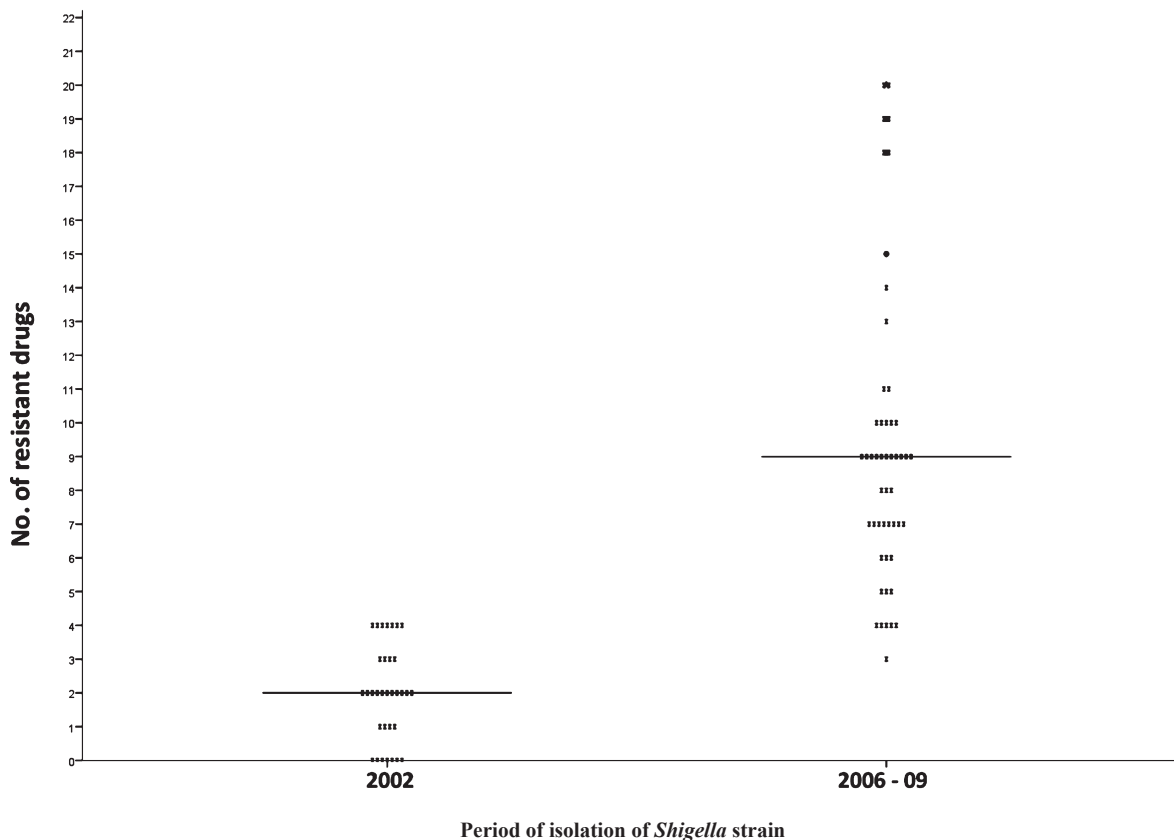


Fig. Distribution of *Shigella* isolates in 2002 and 2006-2009 by number of antimicrobial the isolate was resistant to.

acid became the mainstay of antibacterial therapy in shigellosis. Resistance to this drug was first reported in these islands in late 90s¹³ and by 2006-2009 almost all the isolates became resistant to it. Other quinolones such as norfloxacin, ciprofloxacin and ofloxacin then became the primary choice for antibacterial therapy. Although fluoroquinolones are recommended as the drugs of choice for shigellosis by World Health Organization¹⁴, emergence of fluoroquinolone resistance among *Shigella* spp. has now been documented in many countries¹⁷⁻¹⁹. At present, alternate drugs such as the third generation cephalosporins are being used commonly. The present study shows that *Shigella* strains are rapidly acquiring resistance to these drugs as well. The emergence of plasmid borne resistance to these cephalosporins further reduces the choice of drugs for the treatment of shigellosis. The genetic transfer of drug resistance genes may not be of immediate concern for the treating clinicians, but will pose a potential problem in the future. ESBLs have evolved greatly over the last 20 yr. Their presence, plus the potential for plasmid-mediated quinolone and carbapenem resistance, will be sure to create significant therapeutic problems in the future.

A larger proportion of *S. dysenteriae*, which is usually the cause of outbreaks and severe disease, is resistant to multiple drugs as compared to other *Shigella* spp. In case of any future outbreak of *S. dysenteriae* in the islands the clinicians would be left with little choice of antimicrobial drugs to treat patients.

Widespread selective pressure and efficient dissemination channels for multi-drug resistant organisms are major factors that might have contributed to the rapid emergence and spread of resistant organisms²⁰.

In conclusion, the emergence of resistance to several new drugs such as fluoroquinolones, 3rd generation cephalosporins, and macrolides in *Shigellae* is a cause of great concern not only at local level but at regional level also. A comprehensive strategy for resistance control involving regulation of drug availability, antimicrobial drug quality assurance, adequate surveillance and discouraging the culture of antimicrobial abuse needs to be evolved²¹. A network of laboratories for real-time monitoring of antibiotic resistance among enteric pathogens and timely dissemination of such information to the clinicians for modification of treatment strategy is the need of the hour.

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