Characterization of non-typhoidal *Salmonella* isolates from children with acute gastroenteritis, Kolkata, India, during 2000–2016

Priyanka Jain¹ · Goutam Chowdhury¹ · Sriparna Samajapati¹ · Surajit Basak² · Arindam Ganai¹ · Sandip Samanta³ · Keinosuke Okamoto⁴ · Asish K. Mukhopadhyay¹ · Shanta Dutta¹

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Abstract

Non-typhoidal *Salmonella* (NTS) is an important cause of acute gastroenteritis in children. The study was undertaken to determine the isolation rate, serovar prevalence, antimicrobial resistance (AMR) profiles, and molecular subtypes of NTS from a hospital-based diarrheal disease surveillance in Kolkata, India. Rectal swabs were collected from children (< 5 years of age) with acute gastroenteritis from 2000 to 2016. Samples were processed following standard procedures for identification of NTS. The isolates were tested for antimicrobial susceptibility, AMR genes, plasmid profiles, multilocus sequence typing (MLST), and pulsed-field gel electrophoresis (PFGE) subtypes. A total of 99 (1.0%) *Salmonella* isolates were recovered from 9957 samples processed. Of the 17 *Salmonella* serovars identified, *S. Worthington* (33%) was predominant followed by *S. Enteritidis* (13%), *S. Typhimurium* (12%), and others. The isolates showed high resistance towards nalidixic acid (43%), ampicillin (34%), third-generation cephalosporins (32%), and azithromycin (25%), while low resistance was observed for fluoroquinolones (2%). Extended-spectrum beta-lactamase production (*bla*_{CTX-M-15} and *bla*_{SHV-12} genes) and azithromycin resistance (*mphA* gene) were common in *S. Worthington*, while fluoroquinolone resistance (*gyrA* and *parC* mutations) was found in *S. Kentucky*. Diverse plasmid profiles were observed among the isolates. PFGE analysis identified genetically related strains of each serovar in circulation. MLST also revealed phylogenetically clonal isolates of which *S. Worthington* ST592 and ciprofloxacin-resistant *S. Kentucky* ST198 were not reported earlier from India. NTS resistant to current drugs of choice poses a potential public health problem. Continuous monitoring of AMR profiles and molecular subtypes of NTS serovars is recommended for controlling the spread of resistant organisms.

Keywords Non-typhoidal *Salmonella* · Gastroenteritis · Children · Antimicrobial resistance · ESBL · MLST · PFGE

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✉ Shanta Dutta
shanta1232001@yahoo.co.in

- ¹ Division of Bacteriology, ICMR-National Institute of Cholera and Enteric Diseases, P-33 CIT Road, Scheme XM, Beliaghata, Kolkata 700010, India
- ² Division of Bioinformatics, ICMR- National Institute of Cholera and Enteric Diseases, Kolkata, India
- ³ Department of Pediatrics, Dr. B. C. Roy Post Graduate Institute of Paediatric Sciences, Kolkata, India
- ⁴ Collaborative Research Centre of Okayama University for Infectious Diseases at NICED, Kolkata, India

Introduction

Gastroenteritis is one of the leading causes of morbidity among the children throughout the world, especially in developing countries with an estimated 1.87 million deaths annually in children < 5 years of age [1]. In India, diarrhea is third leading cause of childhood mortality and responsible for 13% of all deaths per year in children under 5 years [2]. Among the bacterial etiology of diarrhea, non-typhoidal *Salmonella* (NTS) is one of the major zoonotic pathogens reported in children, transmitted by ingestion of contaminated food or water and by contact with animals or poultry [3–5]. Among more than 2500 *Salmonella* serovars, *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) and *S. Enteritidis* are two most common serotypes associated with NTS gastroenteritis in children across the globe [5–13]. Low immunity, immature endogenous bowel flora, and gastric hypochlorhydria

children predispose to NTS infection [5, 13, 14]. NTS gastroenteritis is usually self-limiting and may be accompanied by fever, abdominal cramps, nausea, and vomiting. Hospitalization may be required for severe cases [5]. The global burden of NTS gastroenteritis is high with around 93.8 million cases and 155,000 deaths reported each year [5, 13]. Bacteremia, meningitis, osteomyelitis, and pneumonia are the most common systemic complications reported in 2–47% children with NTS gastroenteritis. Chronic carriage (excretion of NTS > 1 year) is common in children < 5 years which play an important role in spread of the organism in the community and maintain a reservoir of infection [5, 14]. Several foodborne and nosocomial outbreaks of gastroenteritis have been reported among children [15–18].

Antimicrobial therapy is indicated only for severe or invasive salmonellosis. Indiscriminate use of antimicrobials in both humans and veterinary has led to emergence of antimicrobial resistance (AMR) to both conventional (ampicillin, chloramphenicol, tetracycline, and co-trimoxazole) and newer (third-generation cephalosporins, fluoroquinolones, and azithromycin) drugs in NTS, which is a global concern [4, 6–13, 19–23]. Since the resistance rate in NTS varies with different serotypes and in various geographical regions, knowledge on prevalent serovars and their AMR profiles is useful in formulating appropriate treatment (empirical) guidelines and controlling spread of the organisms in the community. Mobile genetic elements like plasmids and integrons harboring resistance genes play an important role in AMR dissemination. Among these, Class 1 integron is the common type found in antibiotic-resistant NTS and reported from different countries [24–27].

Molecular subtyping of bacteria is an invaluable tool for determining relatedness among the bacterial isolates in epidemiological surveillance and in outbreak investigation [28, 29]. Plasmid profiling is a common typing method used for discriminating *Salmonella* serovars and subtypes within the serovars. It is also useful in tracking the transmission of AMR genes among bacteria. However, the method has low reproducibility and is not suitable for typing plasmid-less bacteria [9, 28]. Pulsed-field gel electrophoresis (PFGE) is considered as gold standard for *Salmonella* subtyping but standardization of protocol is necessary to ensure reproducibility. Multilocus sequence typing (MLST) is a DNA sequence-based method using several housekeeping genes which is more suitable in studying the evolutionary relationships among the isolates. The MLST data is unambiguous, stable, and easily comparable among various labs [28–30].

Epidemiology of NTS gastroenteritis in children is far less documented in India due to lack of an effective surveillance in place and lack of suitable diagnostics. In view of the importance of NTS in childhood diarrhea, this study was undertaken to determine the isolation rate, predominant serotypes, AMR patterns, plasmid profiles, and molecular subtypes by MLST and PFGE of NTS isolates from hospital-attending children <

5 years of age with acute gastroenteritis in Kolkata, India, during 2000–2016.

Materials and methods

Sample collection

Rectal swabs were collected from children < 5 years of age, attending the diarrheal treatment unit (DTU) at the OPD of Dr. B.C Roy Post Graduate Institute of Paediatric Sciences (a government pediatric hospital in West Bengal), Kolkata, India, from January 2000 to December 2016. Patients with acute diarrhea (defined as passage of 3 or more loose or liquid stools per day or more frequently than is normal for the individual as per World Health Organization guidelines) [31], and without prior antibiotic treatment were eligible for inclusion in the study. Samples were collected from every fifth patient from Monday to Friday (except government holidays) between 10.00 am to 1.00 pm. On enrollment, a detailed clinical history regarding the nature, frequency, and duration of diarrhea, frequency of vomiting, abdominal pain, pyrexia, and degree of dehydration were recorded in a standard pro forma. Rectal swabs were collected in Cary Blair's transport medium and submitted to the Bacteriology division of ICMR-National Institute of Cholera and Enteric Diseases (NICED) within 2 h of collection for isolation of enteric bacterial pathogens including *Salmonella* spp.

Sample processing and identification of *Salmonella* spp.

The isolation and identification of *Salmonella* spp. was done following standard microbiological methods [32]. Briefly, the rectal swab were inoculated onto two different selective media, namely, MacConkey Agar (MAC) and Hektoen Enteric Agar (HEA) (Difco, MD, USA) both directly as primary culture and following growth in Selenite F enrichment broth (for 12 h at 37 °C). Both primary and secondary culture plates were incubated at 37 °C for 18–24 h.

The presumptive identification of *Salmonella* spp. was based on colony morphology and standard biochemical tests [33]. Strain identification was confirmed by serotyping using *Salmonella* poly- and monovalent O and H antisera (Denka Seiken Pvt. Ltd., Tokyo, Japan). The *Salmonella* serotype was determined following White-Kauffmann-Le Minor scheme [34]. All *Salmonella* isolates were preserved at – 80 °C in sterile Trypticase soy broth with 50% glycerol for further studies.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test of the studied isolates was done following Kirby-Bauer disc diffusion method on Mueller Hinton agar (Merck, Darmstadt, Germany) using 17

antibiotic discs (BD BBL™, Maryland, USA): ampicillin (Amp), chloramphenicol (Chl), tetracycline (Tet), co-trimoxazole (Sxt), nalidixic acid (Na), ciprofloxacin (Cip), norfloxacin (Nor), ofloxacin (Ofx), gentamicin (Gm), amikacin (An), streptomycin (Str), cefotaxime (Ctx), ceftazidime (Caz), ceftriaxone (Cro), aztreonam (Atm), azithromycin (Azm), and amoxycillin-clavulanic acid (Amc). Minimum inhibitory concentrations (MICs) of the antimicrobials for the resistant isolates were determined by E-test (AB Biodisk, Solna, Sweden). Results were interpreted following Clinical and Laboratory Standards Institute guidelines (CLSI) [35]. *Escherichia coli* ATCC 25922 was used as control. No Azm MIC breakpoint has been defined for NTS by CLSI. However, researchers have proposed 16 µg/ml as “epidemiological cut-off” value for wild type *Salmonella* spp. [22, 36]. Phenotypic detection of extended-spectrum β-lactamase (ESBL) was done by cephalosporin/clavulanate combination disc test [37].

Determination of AMR genes, integrons, and their transferability

The studied isolates were screened by PCR for presence of AMR genes like *catA* and *cmlA* (chloramphenicol resistance); *tetA* and *tetB* (tetracycline resistance); *dfrIa*, *dfrVII*, *dfrXII*, *sulI*, *sul2*, and *sul3* (co-trimoxazole resistance); *strA*, *strB*, and *aadA* (streptomycin resistance); *aac(6′)-Ib*, *aadB*, and *grm* for other aminoglycosides like amikacin and gentamicin; *bla_{TEM}*, *bla_{SHV}*, *bla_{CTX-M}*, and *bla_{OXA}* (for ESBLs); and *mphA* and *mphB* (azithromycin resistance). Quinolone-resistant isolates were tested for any chromosomal mutations in the *gyrA*, *gyrB*, *parC*, and *parE* genes of quinolone-resistance-determining regions (QRDRs) and for presence of plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*, and *qepA*. Presence of class 1 integron (*int1*) along with gene cassettes was also determined using published primer sequences and PCR conditions [37–41]. Each PCR was followed by direct sequencing of the PCR product using 3730 DNA Analyzer (Applied Biosystems, Foster City, CA, USA). Sequences obtained were analyzed by comparison with the National Centre for Biotechnology Information (NCBI) database sequences using BLAST program.

Conjugation experiment was attempted by broth mating procedure in Tryptic soy broth (Difco), using sodium azide-resistant *E. coli* J53 as the recipient strain and resistant *Salmonella* strains as donors [42]. Transconjugants were selected as lactose-fermenting colonies of *E. coli* on MacConkey agar supplemented with sodium azide (100 µg/ml) and one of the following antibiotics: chloramphenicol (16 µg/ml) or cefotaxime (4 µg/ml). The transfer of AMR in transconjugants was checked by disc diffusion, MIC by E-test, and presence of respective resistance genes by PCR.

Plasmid isolation and incompatibility typing

Plasmid DNA was isolated from the studied isolates and transconjugants following Kado and Liu method [43]. Plasmids were electrophoretically separated on 0.8% agarose gel at 70 V for 3 h in Tris-acetic acid-EDTA buffer and visualized after staining with ethidium bromide (0.5 µg/ml) solution. The plasmid size was determined by Quantity One software version 4.5 (Bio-Rad) using *E. coli* V517 and/or *Shigella flexneri* YSH6000 strains as molecular weight markers. The incompatibility types of the plasmids were determined by PCR using published suitable primers [44].

MLST

MLST was performed by PCR amplification followed by sequencing of the seven housekeeping genes: *aroC*, *dnaN*, *hemD*, *hisD*, *purE*, *sucA*, and *thrA* as described earlier [30]. The sequence type (ST) for each isolate was obtained on the basis of the sequences of the alleles of aforesaid genes (https://enterobase.warwick.ac.uk/species/senterica/allele_st_search).

PFGE

PFGE of *Xba*I-digested genomic DNA of the studied isolates was performed according to the PulseNet standard protocol using CHEF DRIII apparatus (Bio-Rad) [45]. *S. Braenderup* H9812 was used as reference strain. The PFGE profiles were analyzed using FP Quest™ software version 4.5 (Bio-Rad). Similarity analysis was determined by Dice coefficient and clustering of bands was done by unweighted pair group method with arithmetic mean (UPGMA). The isolates with identical PFGE patterns (100% similarity) were described as clonal. Considering that the strains included in this study were sporadic in nature and isolated over a long period of time from 2000 to 2016, the isolates with PFGE patterns differing by three or less bands (≥ 80% similarity) were designated as closely related or probably (possibly) related; isolates differing by four or more bands (< 80%) in PFGE profiles were considered as unrelated [46].

The dendrogram obtained in PFGE was further validated statistically by maximum likelihood (ML) algorithm. The PFGE data set of 82 isolates were compiled into character matrix and subjected to ML analysis available in PAUP* (phylogenetic analysis using parsimony) version 4.0a166 (<http://phylosolutions.com/paup-test>). The resulting tree was subjected to 100 bootstrap replicates [47].

Statistical analysis

All statistical analyses were done using Epi Info™ software for Windows (Centers for Disease Control and Prevention, Atlanta, GA, USA). Pearson’s chi-squared test (χ^2) was used

to evaluate possible correlation between NTS isolation and seasons; NTS isolation and demographic & clinical characteristics of patients; and NTS serotypes and AMR. All statistical tests in this study were two-sided and p values < 0.05 were considered statistically significant.

Results

Isolation rate and seasonal variation of *Salmonella* gastroenteritis

A total of 9957 rectal swabs were collected from children < 5 years of age with acute diarrhea during 17 years period from January 2000 to December 2016 and processed. A total of 99 (0.99%) *Salmonella* isolates were recovered. These included 97 NTS isolates and one isolate each of *S. Typhi* and *S. Paratyphi A*. The isolation rate of *Salmonella spp.* over the period of 17 years is shown in Fig. 1. *Salmonella* isolation was significantly ($p = 0.01$) associated with the summer (Mar–May) ($n = 14/1095$; 1.28%) and monsoon (June–Sept) ($n = 52/4277$; 1.22%) months as compared to autumn (Oct–Nov) ($n = 13/1193$; 1.09%) and winter (Dec–Feb) ($n = 20/3392$; 0.59%) months (Fig. 2).

Clinical features of patients with *Salmonella* gastroenteritis

The demographic and clinical characterization data of patients with acute diarrhea enrolled in this study is shown in Table 1. Children aged 12–23 months ($n = 30/2210$; 1.36%) showed significantly ($p < 0.01$) higher isolation of *Salmonella spp.* than children aged 0–11 months ($n = 49/4350$; 1.13%) and 24–59 months ($n = 20/3397$; 0.59%). Male children ($n = 55/5054$; 1.08%) were affected marginally more than the female

children ($n = 44/4903$; 0.89%), ($p > 0.05$). The most common type of diarrhea observed in patients with *Salmonella* infection was watery diarrhea ($n = 54/99$, 54.5%) followed by diarrhea containing mucus (24.2%) and diarrhea with blood-mucus (21.2%). The frequency of stool passage on an average was 6 times per day. Majority of the patients had no dehydration (71.7%), abdominal pain (83.8%), fever (62.6%), or vomiting (75.8%).

Salmonella serotypes

A total of 17 serovars belonging to eight serogroups were identified among the 99 isolates (Table S1). *S. Worthington* ($n = 33$; 33%) was the predominant, followed by *S. Enteritidis* ($n = 13$; 13%), *S. Typhimurium* ($n = 12$; 12%), *S. Bareilly* ($n = 9$; 9%), *S. Virchow* ($n = 7$; 7%), *S. Weltevreden* ($n = 6$; 6%), *S. Infantis*, *S. Newport* ($n = 3$; 3% each), *S. Agona*, *S. Kentucky*, *S. Litchfield*, *S. Paratyphi B* var. Java ($n = 2$, 2% each), *S. Brunei*, *S. Chester*, *S. Paratyphi A*, *S. Teko*, and *S. Typhi* ($n = 1$, 1% each). The stack bar diagram shows the percentage distribution of *Salmonella enterica* serovars in every year (Fig. 3).

Antimicrobial resistance and MIC

The percentage of AMR in *Salmonella* fecal isolates and MIC towards the antibiotics is given in Table 2. Overall 55.6% of the isolates were pansusceptible (defined as susceptible to all 17 antimicrobials tested in this study), while 44.4% isolates showed resistance to at least one antimicrobial (Table 2). The common antimicrobial to which most of the isolates of *Salmonella spp.* showed resistance was Na (43.4%). Resistance to first-line drugs used for treatment of salmonellosis were 34.3%, 7.1%, 6.1%, and 1.0% respectively towards Amp, Sxt, Tet, and Chl. Among the current choice of drugs,

Fig. 1 Year-wise distribution (%) of *Salmonella enterica* isolated from rectal swabs of children with acute diarrhea in Kolkata, India, from 2000 to 2016

Fig. 2 Seasonal distribution (%) of *Salmonella enterica* isolated from rectal swabs of children with acute diarrhea in Kolkata, India, during 2000–2016

high resistance of 32.3% and 25.3% was observed against third-generation cephalosporins (3GCs; Caz, Ctx, and Cro) and Azm respectively, while low resistance of 2% was observed against fluoroquinolones (FQs; Cip, Ofx, and Nor). All (100%) isolates were susceptible to Amc. Multidrug resistance (resistance to ≥ 3 antimicrobial classes) was observed among 37.4% isolates spanning over five serovars *S. Worthington* ($n = 32$, 86.5%), *S. Kentucky* ($n = 2$, 5.4%), *S. Typhimurium*, *S. Infantis*, and *S. Virchow* ($n = 1$, 2.7% each). Among all serotypes, AMR was significantly associated with *S. Worthington* ($p < 0.001$) (Table 2).

Antimicrobial resistance profiles and genes mediating antimicrobial resistance

Twelve AMR-profiles (I–XII) were observed among resistant *Salmonella* studied isolates (Table 3). The profile V (Na^R) was common among *S. Worthington*, *S. Typhimurium*, *S. Infantis*, and *S. Typhi* ($n = 1$ each). Presence of AMR genes, chromosomal mutations associated with Na resistance, and class 1 integron found among the studied isolates is shown in Table 3.

Plasmid characterization

One or more plasmids of molecular size ranging from 4 to 254 kb were found in 65 (65.7%) studied isolates (Table 4). Diversity in plasmid profiles was found both across the serovars and within each serovar, with a total of 31 plasmid profiles (P1–P31). No correlation between plasmid profiles and AMR profiles was observed. Plasmids were present in both resistant ($n = 36/65$, 55.4%) and pansusceptible ($n = 29/65$; 44.6%) isolates. Among plasmid-less isolates ($n = 34$), 26 were pansusceptible and eight [*S. Infantis* ($n = 3$), *S. Kentucky* ($n = 2$), *S. Virchow* ($n = 2$), and *S. Typhi* ($n = 1$)] were resistant.

The plasmid incompatibility typing revealed presence of IncFIB ($n = 49$, 75.4%), IncI1 ($n = 32$, 49.2%), IncFIA ($n =$

31, 47.7%), IncFIIS ($n = 30$, 46.2%), and IncFIIA ($n = 1$, 1.5%) types among 65 studied isolates (Table 4). In two isolates, plasmids were untypable. IncFIA and IncI1 type plasmids were found only in *S. Worthington* isolates, whereas IncFIIS type plasmid was found in all serovars except *S. Worthington*.

MLST and PFGE analysis

MLST and PFGE were performed for 85 isolates comprising eight important serovars. The *S. Worthington*, *S. Bareilly*, *S. Virchow*, *S. Weltevreden*, *S. Infantis*, and *S. Kentucky* isolates were assigned to sequence types ST592, ST203, ST16, ST365, ST32, and ST198 respectively. ST36 and ST19 were found in *S. Typhimurium* and ST11 and ST1975 types were common in *S. Enteritidis* isolates (Fig. 4). Sequence type (ST592) of *S. Worthington* was not reported earlier from India.

PFGE generated 63 pulsotypes (PP) and grouped *Salmonella* isolates into eight distinct clusters A, B, C, D, E, F, G, and H which were serovar-specific and represented Enteritidis ($n = 13$), Weltevreden ($n = 6$), Typhimurium ($n = 12$), Bareilly ($n = 6$), Kentucky ($n = 2$), Worthington ($n = 31$), Virchow ($n = 7$), and Infantis ($n = 3$) respectively. However, two *S. Worthington* isolates (pulsotypes PP22 and PP30) did not belong to cluster F. Three *S. Bareilly* isolates remained untypable by *Xba*I enzyme. With the exception of *S. Virchow*, strains belonging to serovars Enteritidis, Weltevreden, Typhimurium, Bareilly, Kentucky, Worthington, and Infantis were found to be closely related or probably related ($> 80\%$ similarity coefficient). Some of the strains within each cluster were found to be clonal in nature (100% similarity coefficient) indicating origin from same source. The *S. Virchow* strains although found to be genetically unrelated (76.6% similarity coefficient), yet few clonal or closely related strains (PP55, PP56, PP58, PP59)

Table 1 Demographic and clinical characterization data of patients ($n = 9957$) with acute diarrhea enrolled in this study

Sl. no.	Factors	Total	No. (%) of <i>Salmonella</i> positives	χ^2	p value ^a
A. Demographic characteristics					
1.	Age				
	0–11 months	4350	49 (1.13)	9.41	< 0.01 (S)
	12–23 months	2210	30 (1.36)		
	24–59 months	3397	20 (0.59)		
2.	Gender			0.92	> 0.05 (NS)
	Male	5054	55 (1.08)		
	Female	4903	44 (0.89)		
3.	Family income (Rs)			4.9	> 0.05 (NS)
	< 3000	578	10 (1.73)		
	3001–5000	4903	51 (1.04)		
	5001–10,000	3761	34 (0.90)		
	> 10,000	715	4 (0.56)		
B. Clinical characteristics					
1.	Type of diarrhea			17.95	< 0.001 (S)
	Watery diarrhea	3431	54 (1.57)		
	Diarrhea with mucus	3310	24 (0.73)		
	Diarrhea with blood-mucus	3216	21 (0.65)		
2.	Frequency of stool passage per day			12.74	0.0017 (S)
	3–4 times	3297	21 (0.64)		
	5–6 times	4289	60 (1.39)		
	> 6times	2371	18 (0.76)		
3.	Degree of dehydration			26.53	< 0.0001 (S)
	No	4952	71 (1.43)		
	Some	2796	25 (0.89)		
	Severe	2209	3 (0.14)		
4.	Fever			5.25	0.021 (S)
	Present	4862	37 (0.76)		
	Absent	5095	62 (1.22)		
5.	Abdominal pain			66.28	< 0.0001 (S)
	Present	5628	16 (0.28)		
	Absent	4329	83 (1.92)		
6.	Vomiting			13.54	0.00023 (S)
	Present	4225	24 (0.56)		
	Absent	5732	75 (1.31)		

^a S significant, NS not significant

were also found within the cluster G (Fig. 4). The different PFGE clusters specific for different serovars suggested the presence of diverse reservoirs of NTS infection in Kolkata. However, the circulation of closely related or probably related strains of each serotype within each cluster speculated towards diversification of serotype-specific strains from a common reservoir of infection over a long period of time.

Maximum likelihood analysis of the PFGE profiles of NTS isolates also showed serovar-specific clustering (Fig. S1). The designation of the clusters (A–H) representing serovars Enteritidis, Weltevreden, Typhimurium, Bareilly,

Kentucky, Worthington, Virchow, and Infantis respectively has been retained as in Fig. 4 for easy understanding. Except for the subtle positional change in clustering of C (S. Typhimurium) and D (S. Bareilly) & G (S. Virchow) and H (S. Infantis), the overall clustering of strains by ML method did not change. Thus, phylogenetic analysis by PFGE showed genetically related strains in circulation among children in Kolkata, India.

The characteristics of *S. Typhimurium* fecal isolates described in this study were partially published in our earlier study [42].

*Others include serovars Teko (5%; 2007), Agona (10%; 2007), Litchfield (8.3% in 2014; 10% in 2015), Brunei (10%; 2015) and Chester (12.5%; 2016).

Fig. 3 Year-wise distribution (%) of *Salmonella enterica* serovars isolated from rectal swabs of children with acute diarrhea in Kolkata, India, from 2000–2016.

Discussion

In the present study, the rate of isolation of *Salmonella* in children < 5 years of age with acute diarrhea in and around Kolkata over 17 years (2000–2016) was found to be 1%, which was considerably less compared to an earlier study (15.3%) from Kolkata (1993–1996) in children ≤ 2 years of age [6]. During the studied period, the isolation rate of *Salmonella* in the year 2007 was found to be unusually high (4.9%) in comparison with other years (Fig. 1). This could be due to the disastrous rainfall and flood that severely affected areas in and around Kolkata during 2007 [48]. A decrease in isolation of *Salmonella* spp. from 3.9% (1994–1998) to 1% (2002–2010) was also noted and reported among gastroenteritis patients of all age group from North India [49]. The reason behind the decline in isolation rate of NTS in recent years was not obvious, but could be due to the awareness development campaign by the Government of India for access to safe and clean potable water, for improved sanitation, and hygiene practice. The prevalence rate of *Salmonella* in stool samples of under 5 years children as reported from different countries was 1.9% (Nepal), 4.3–17.2% (China), 5.4% (Vietnam), 10.3% (Iraq), and 2.5–25.5% (African countries) [4, 10–14, 50–54]. The wide variation in isolation rate of enteric *Salmonella* strains may be attributable to difference in local

geography, socioeconomic conditions, season, and duration of sampling as well as access to medical facility and laboratory testing.

In this study, cases of *Salmonella* gastroenteritis peaked in the summer and monsoon months (Mar–Sept) ($p = 0.01$) (Fig. 2), which was consistent with earlier studies from China, India, Iraq, and Vietnam [4, 6, 13, 14]. Hot and humid conditions facilitate microbial replication leading to food contamination and transmission of pathogens causing foodborne diseases.

Children aged 12–23 months ($p < 0.01$) were found to be significantly more susceptible to *Salmonella* gastrointestinal (GI) infection. Similar results were reported from China, Iraq, and Zambia [4, 13, 54]. This age group is more vulnerable to GI infection due to reduced protective immunity caused by switch from breast to bottle feeding, habits of crawling, and tendency to put fingers or fomites inside the mouth. Regarding clinical manifestation, half of the studied children (54.5%) presented with watery diarrhea which was consistent with reports from Iraq (75.8%) and Vietnam (54%) [13, 14]. Unlike other studies [13, 14], abdominal pain and fever were not reported to be the cardinal features of *Salmonella* gastroenteritis in the current study.

In this study, *S. Worthington*, *S. Enteritidis*, *S. Typhimurium*, *S. Bareilly*, *S. Virchow*, and *S. Weltevreden* were the frequently

Table 2 Percentage of antimicrobial resistance (AMR) and the minimum inhibitory concentrations (MIC) range of antimicrobials in *Salmonella enterica* fecal isolates ($n = 99$)* from children in Kolkata (2000–2016)

Antimicrobials (disc potency in μg) ^a	MIC ($\mu\text{g/ml}$)	Total no. (%) of AMR	Distribution of AMR in <i>Salmonella enterica</i> serovars
Na (30)	> 256	43 (43.4)	Worthington ($n = 33$); Infantis ($n = 3$); Kentucky ($n = 2$); Typhimurium ($n = 2$); Paratyphi A ($n = 1$); Typhi ($n = 1$); Virchow ($n = 1$)
Amp (10)	> 256	34 (34.3)	Worthington ($n = 32$); Kentucky ($n = 2$)
Caz (30)	16–256	32 (32.3)	Worthington ($n = 32$)
Ctx (30)	> 32	32 (32.3)	Worthington ($n = 32$)
Cro (30)	> 256	32 (32.3)	Worthington ($n = 32$)
Atm (30)	> 256	32 (32.3)	Worthington ($n = 32$)
Str (10)	64–128	30 (30.3)	Worthington ($n = 26$); <i>S. Kentucky</i> ($n = 2$); <i>S. Infantis</i> ($n = 1$); <i>S. Typhimurium</i> ($n = 1$)
An (30)	64–128	27 (27.3)	Worthington ($n = 27$)
Azm (15)	32–128	25 (25.3)	Worthington ($n = 24$); Paratyphi A ($n = 1$)
Sxt (25)	> 32	7 (7.1)	Infantis ($n = 2$); Kentucky ($n = 2$); Virchow ($n = 2$); Typhimurium ($n = 1$)
Tet (30)	48–> 256	6 (6.1)	Kentucky ($n = 2$); Virchow ($n = 2$); Infantis ($n = 1$); Typhimurium ($n = 1$)
Cip (5)	8	2 (2.0)	Kentucky ($n = 2$)
Nor (10)	16	2 (2.0)	Kentucky ($n = 2$)
Oxf (5)	32	2 (2.0)	Kentucky ($n = 2$)
Gm (10)	16	2 (2.0)	Kentucky ($n = 2$)
Chl (30)	> 256	1 (1.0)	Typhimurium ($n = 1$)
Resistance to 0 antimicrobial (pansusceptible)		55 (55.6)	Enteritidis ($n = 13$); Typhimurium ($n = 10$); Bareilly ($n = 9$); Weltevreden ($n = 6$); Virchow ($n = 5$); Newport ($n = 3$); Agona ($n = 2$), Litchfield ($n = 2$); Paratyphi B var. Java ($n = 2$); Brunei ($n = 1$); Chester ($n = 1$); Teko ($n = 1$)
Resistance to 1 antimicrobial	–	4 (4.0)	Infantis ($n = 1$); Typhimurium ($n = 1$); Virchow ($n = 1$); Worthington ($n = 1$)
Resistance to 2 antimicrobials	–	3 (3.0)	Infantis ($n = 1$); Paratyphi A ($n = 1$); Virchow ($n = 1$)
Resistance to ≥ 3 antimicrobials	–	37 (37.4)	Worthington ($n = 32$); Kentucky ($n = 2$); Infantis ($n = 1$); Virchow ($n = 1$); Typhimurium ($n = 1$)

*Fifty-five (55.6%) isolates were pansusceptible (susceptible to all 17 antimicrobials tested in the study) while 44 (44.4%) isolates showed resistance to one or more above tested antimicrobials

^aAmp ampicillin, An amikacin, Atm aztreonam, Azm azithromycin, Caz ceftazidime, Chl chloramphenicol, Cip ciprofloxacin, Cro ceftriaxone, Ctx cefotaxime, Gm gentamicin, Na nalidixic acid, Nor norfloxacin, Oxf ofloxacin, Str streptomycin, Sxt co-trimoxazole, Tet tetracycline

isolated serovars. The WHO Global Foodborne Infections Network's report on NTS surveillance from both developed and developing countries showed *S. Enteritidis* and *S. Typhimurium* to be the predominant serovars isolated from humans [55]. *S. Virchow* was ranked among the top 15 serovars in Asia and Europe. Serovars like *S. Worthington*, *S. Weltevreden*, and *S. Bareilly* were commonly reported from South Asian countries including India [55, 56]. Presence of a local animal reservoir or food greatly contributed towards dominance of certain serotypes in particular regions [55]. Interestingly, *Salmonella* serotype distribution at one place may vary over a course of time. In this study, *S. Worthington* dominated during 2000–2005 and was then gradually replaced by other serovars since 2007 and *S. Typhimurium* emerged as

the most prevalent serovar during 2013–2016 (Fig. 3). The serovar shift from *S. Typhimurium* (1994–1998) to *S. Senftenberg* (2002–2010) was reported in North India [49]. Bassal et al. reported predominance of *S. Infantis* over *S. Enteritidis* (1999–2008) in Israel post 2008 [57]. The shift from *S. Enteritidis* (2010) to *S. Typhimurium* (2011–2015) as the predominant serovar among humans was also reported from southern Brazil [58]. Changes in meteorology, human food habits, international travel, and trade have been associated with the temporal dominance of certain serotypes followed by a decline and replacement with another [52, 57].

Antimicrobial resistance in *Salmonella* is a growing public health problem. Resistance to first-line drugs (Amp, Chl, Tet, and Sxt) was found to be considerably lower (1–34%)

Table 3 Phenotypic and genetic profiles of antimicrobial resistance (AMR) of *Salmonella enterica* fecal isolates ($n = 44$)* from Kolkata during 2000–2016

Serotype	AMR profile (<i>n</i>) ^a	Detection of AMR genes					Mutations in QRDR ^b					Class 1 integron		
		Amp	Caz, Ctx, Cro, Atm	Azm	Str	An	Gm	Chl	Tet	Sxt	<i>gyrA</i>	<i>parC</i>	Approximate size of gene cassette	Gene cassette array
<i>S. Worthington</i>	I	Na, Amp, Caz, Ctx, Cro, Atm, An, Str, Azm (24)	<i>bla</i> _{TEM-1}	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-12}	<i>mphA</i>	<i>aadA1</i>	<i>aac(6')-Ib</i>	–	–	–	S83Y [TCC → TAC]	–	–	–
	II	Na, Amp, Caz, Ctx, Cro, Atm, An, Str (2)	<i>bla</i> _{TEM-1}	<i>bla</i> _{CTX-M-15}	–	<i>aadA1</i>	<i>aac(6')-Ib</i>	–	–	–	S83Y [TCC → TAC]	–	–	–
	III	Na, Amp, Caz, Ctx, Cro, Atm, An (1)	<i>bla</i> _{TEM-1}	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-12}	–	–	<i>aac(6')-Ib</i>	–	–	–	S83Y [TCC → TAC]	–	–	–
	IV	Na, Amp, Caz, Ctx, Cro, Atm (5)	<i>bla</i> _{TEM-1}	<i>bla</i> _{CTX-M-15} , <i>bla</i> _{SHV-12}	–	–	–	–	–	–	S83Y [TCC → TAC]	–	–	–
	V	Na (1)	–	–	–	–	–	–	–	–	–	S83Y [TCC → TAC]	–	–
<i>S. Infantis</i>	VI	Tet, Sxt, Str, Na (1)	–	–	–	<i>aadA1</i>	–	–	<i>tetA</i>	<i>sul1</i>	D87Y [GAC → TAC]	–	1.0 kb	<i>aadA1</i>
	VII	Sxt, Na (1)	–	–	–	–	–	–	–	<i>dfiA1</i> , <i>sul1</i>	D87Y [GAC → TAC]	–	1.2 kb	<i>dfiA1-orfC</i>
<i>S. Kentucky</i>	V	Na (1)	–	–	–	–	–	–	–	–	D87Y [GAC → TAC]	–	–	–
	VIII	Amp, Tet, Sxt, Str, Gm, Na, Cip, Nor, Ofx (2)	<i>bla</i> _{TEM-1}	–	–	<i>aadA7</i>	<i>aac(3')-Ib</i>	<i>tetA</i>	<i>sul1</i>	S83F [TCC → TTC, D87Y [GAC → TAC]	S80I [AGC → ATC]	1.5 kb	<i>aac(3')-Ib</i> - <i>aadA7</i>	
<i>S. Typhimurium</i>	IX	Chl, Tet, Sxt, Str, Na (1)	–	–	–	<i>aadA1</i> , <i>aadA2</i>	–	–	<i>cmiA1</i>	<i>dfiA12</i> , <i>sul3</i>	D87Y [GAC → TAC]	–	7 kb	<i>dfiA12-orfF-aadA2-cmiA1-aadA1</i>
	V	Na (1)	–	–	–	–	–	–	–	–	D87Y [GAC → TAC]	–	–	–
<i>S. Virchow</i>	X	Tet, Sxt, Na (1)	–	–	–	–	–	–	<i>tetA</i>	<i>sul1</i> , <i>dfiA5</i>	S83Y [TCC → TAC]	–	0.7 kb	<i>dfiA5</i>
<i>S. Paratyphi A</i>	XI	Tet, Sxt (1)	–	–	–	–	–	–	<i>tetA</i>	<i>sul1</i> , <i>dfiA5</i>	–	–	0.7 kb	<i>dfiA5</i>
	XII	Azm, Na (1)	–	–	–	<i>mphA</i>	–	–	–	–	S83 L [TCC → TTG]	–	–	–
<i>S. Typhi</i>	V	Na (1)	–	–	–	–	–	–	–	–	D87N [GAC → AAC]	–	–	–

*Remaining *Salmonella enterica* isolates ($n = 55$) were pansusceptible^a *Amp* ampicillin, *An* amikacin, *Atm* aztreonam, *Azm* azithromycin, *Caz* ceftazidime, *Chl* chloramphenicol, *Cip* ciprofloxacin, *Cro* ceftriaxone, *Ctx* cefotaxime, *Gm* gentamicin, *Na* nalidixic acid, *Nor* norfloxacin, *Ofx* ofloxacin, *Str* streptomycin, *Sxt* co-trimoxazole, *Tet* tetracycline^b QRDR quinolone-resistance-determining region, *S* serine, *T* tyrosine, *F* phenylalanine, *D* aspartate, *L* leucine, *N* asparagine

Table 4 Attributes of plasmids in non-typhoidal *Salmonella enterica* fecal isolates ($n = 65$) from children in Kolkata (2000–2016)

Serotype	Plasmid profiles (kb)	Plasmid type	No. of isolates	AMR-profile ^a	Transconjugant			
					Plasmid profile	Plasmid type	AMR-profile	AMR genes
<i>S.</i> Worthington	P1 79.4, 68.2, 50.8, 4.9	FIA, FIB, II	1	III	79.4, 68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P2 76.4, 68.2, 50.8, 4.9	FIA, FIB, II	2	IV	76.4, 68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
			8	I				
	P3 76.4, 68.2, 50.8	FIA, FIB, II	1	I	76.4, 68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P4 76.4, 68.2, 4.9	FIA, FIB, II	1	I	76.4, 68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P5 76.4, 50.8, 4.9	FIA, FIB, II	1	II	76.4	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P6 70.8, 68.2, 50.8, 4.9	FIA, FIB, II	3	I	70.8, 68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P7 68.2, 62.5, 50.8, 4.9	FIA, FIB, II	1	IV	68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P8 68.2, 50.8, 4.9	FIA, FIB, II	1	IV	68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
			1	II				
<i>S.</i> Typhimurium			10	I				
	P9 68.2, 4.9	I1	1	I	68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P10 68.2	I1	1	IV	68.2	I1	Amp, Caz, Ctx, Cro, Atm	<i>bla</i> _{TEM-1} , <i>bla</i> _{CTX-M-15}
	P11 50.8	FIA, FIB	1	V	—	—	—	—
	P12 254, 130	FIIS, FIB	1	IX	254	FIIS, FIB	Chl, Tet, Sxt, Str	<i>cmlA1</i> , <i>tetA</i> , <i>dfpA12</i> , <i>sul3</i> , <i>aadA1</i> , <i>aadA2</i> , <i>int1</i>
	P13 180, 98.5	FIIS	2	Pansusceptible	—	—	—	—
	P14 130	FIIS, FIB	1	V	—	—	—	—
	P15 108.4	FIIS, FIB	2	Pansusceptible	—	—	—	—
	P16 98.5	FIIS	2	Pansusceptible	—	—	—	—
	P17 98.8	FIIS, FIB	4	Pansusceptible	—	—	—	—
<i>S.</i> Enteritidis	P18 60.4, 52.4, 22.1	FIIS, FIB	1	Pansusceptible	—	—	—	—
	P19 60.4, 45.4	FIIS, FIB	1	Pansusceptible	—	—	—	—
	P20 60.4, 2.6	FIIS, FIB	1	Pansusceptible	—	—	—	—
	P21 60.4	FIIS, FIB	6	Pansusceptible	—	—	—	—
<i>S.</i> Weltevreden	P22 121.4	FIIS	2	Pansusceptible	—	—	—	—
	P23 97.4, 47.5	FIIS	1	Pansusceptible	—	—	—	—
	P24 97.4	FIIS	1	Pansusceptible	—	—	—	—
	P25 83.6, 2.8	FIIS	1	Pansusceptible	—	—	—	—
	P26 83.6	FIIS	1	Pansusceptible	—	—	—	—
<i>S.</i> Agona	P27 5.6	Untypable	1	Pansusceptible	—	—	—	—
<i>S.</i> Paratyphi A	P28 96.2, 4.9	FIIA, FIIS, FIB	1	XII	—	—	—	—
<i>S.</i> Brunei	P29 54.8	FIIS	1	Pansusceptible	—	—	—	—
<i>S.</i> Litchfield	P30 4.0	Untypable	1	Pansusceptible	—	—	—	—
<i>S.</i> Teko	P31 197.8, 97.5	FIIS	1	Pansusceptible	—	—	—	—

^a I, resistant to Na, Amp, Caz, Ctx, Cro, Atm, An, Str, Azm; II, resistant to Na, Amp, Caz, Ctx, Cro, Atm, An, Str; III, resistant to Na, Amp, Caz, Ctx, Cro, Atm, An; IV, resistant to Na, Amp, Caz, Ctx, Cro, Atm; V, resistant to Na; IX, resistant to Chl, Tet, Sxt, Str, Na

Amp ampicillin, An amikacin, Atm aztreonam, Azm azithromycin, Caz ceftazidime, Chl chloramphenicol, Cip ciprofloxacin, Cro ceftriaxone, Ctx cefotaxime, Na nalidixic acid, Str streptomycin, Sxt co-trimoxazole, Tet tetracycline

Dice (Opt 1.50%) (Tol 1.5%-1.5%) (H>0.0% S>0.0%)

Fig. 4 Pulsed-field gel electrophoresis (PFGE) profiles of *XbaI*-digested DNA of non-typhoidal *Salmonella* isolates ($n=82$) from children with acute gastroenteritis, India, 2000–2016. Band comparison was performed using Dice coefficient with 1.50% optimization (Opt) and 1.5% position tolerance (Tol). H minimum height, S minimum surface, Amp ampicillin,

An amikacin, Atm aztreonam, Azm azithromycin, Caz ceftazidime, Chl chloramphenicol, Cip ciprofloxacin, Cro ceftriaxone, Ctx cefotaxime, Gm gentamicin, Na nalidixic acid, Nor norfloxacin, Ofx ofloxacin, Str streptomycin, Sxt co-trimoxazole, Tet tetracycline

(Table 2) in comparison with those reported by Saha et al. (71–99%) in the past (1993–1996) [6]. A decline in resistance to these drugs suggested that these still might be useful choice for empiric treatment. Unlike this study, high resistance rates towards first-line drugs were reported in NTS from countries like China (16–67%), Iraq (50–70%), Nepal (10–70%), Africa (17–100%), and Brazil (15–44%) during the same period [4, 8, 10–13, 50–54, 58, 59]. Among the current choice of drugs, resistance to 3GCs was found in 32% studied isolates, which was not very encouraging as 3GCs are the current drugs of choice for treatment of salmonellosis in children. Although FQ resistance was observed in only 2% of studied isolates,

intermediate susceptibility to Cip (MIC 0.125–0.5 $\mu\text{g/ml}$) and Ofx (MIC 0.25–0.75 $\mu\text{g/ml}$) were found in 41% studied isolates, which was alarming as it has been associated with increased treatment failures with ciprofloxacin in invasive salmonellosis. The rate of resistance to 3GCs (28–42%) and FQs (14–58%) was found to be higher in enteric NTS isolates from children in Asian countries [4, 11–13, 50], than those isolated from children in African countries (0–13% for 3GCs and 0–11% for FQs) [8, 10, 49–52]. Resistance to Azm was found to be 25% in the studied isolates. Although it was lower than those reported from China (44%) and Iraq (67%) [12, 13], judicious use of this drug antimicrobial is recommended as it

is reserved as an alternative antimicrobial for treatment of enteric fever and invasive NTS infections.

With respect to AMR in different serovars, 71.4% *S. Virchow*, 83.3% *S. Typhimurium*, and 100% *S. Enteritidis* studied isolates were pansusceptible, which was in sharp contrast to multiple resistances reported in these serovars worldwide [4, 7–9, 11, 13, 21, 52, 57–59]. All studied isolates of *S. Worthington*, *S. Infantis* and *S. Kentucky* showed resistance to one or more antimicrobial tested (Table 2). High drug resistance was common in these serovars and reported from various countries in the past [10, 23, 49, 52, 60, 61]. ESBL production and FQ resistance were exclusively observed in *S. Worthington* and *S. Kentucky* MDR studied isolates respectively (Table 3).

A single-point mutation (Ser83-Tyr/Leu or Asp87-Tyr/Asn) in *gyrA* gene was found in all Na-resistant isolates of this study. The Cip^R *S. Kentucky* isolates ($n = 2$) possessed double *gyrA* (Ser83-Phe, Asp87Tyr) and a single *parC* (Ser80-Ile) mutation. Similar mutations were reported in various *Salmonella* serovars in the past [23, 27, 38]. The amino acids substitutions at codon 83 or 87 of *gyrA* varied with the serovar (Table 3), which corroborated with the earlier findings of Eaves et al. [38]. ESBL production in *S. Worthington* isolates was mediated by *bla*_{CTX-M-15} ($n = 32/32$; 100%) and *bla*_{SHV-12} ($n = 18/32$; 56.3%) genes leading to resistance against 3GCs and Atm. Earlier studies from India have documented the presence of *bla*_{CTX-M-15}, *bla*_{SHV-12}, and *bla*_{SHV-5} ESBL genes in NTS [27, 49]. The *bla*_{TEM} ESBL genes were not identified in this study, although it was reported from other countries [20]. High-level resistance to Azm in *S. Worthington* (MIC 32–64 µg/ml) and *S. Paratyphi A* (MIC 128 µg/ml) in this study was attributed to the presence of *mphA* gene, which was not reported earlier very frequently. In 2016, Nair et al. from UK showed that *mphA* gene was associated with Azm resistance (MIC ≥ 16 µg/ml) in NTS serovars [22]. Class 1 integron was found in seven (7.1%) isolates (Table 3), of which five were MDR further aggravating the possibility of dissemination of multiple AMR. The gene cassette, found in this study, was widely reported in *Salmonella* serovars from different countries [24–27].

Plasmid analysis was useful in studying the diversity of plasmid profiles within serovars and also to determine the role of plasmid in transmission of AMR to other pathogens. Ten plasmid profiles (P1–P10) were observed in MDR *S. Worthington*, of which P8 and P2 were most common (> 60%) (Table 4). Plasmid of IncII type (79.4, 76.4, 70.8, and 68.2 kb size) was transferable to *E. coli* J53 by conjugation, which carried *bla*_{TEM-1} and *bla*_{CTX-M-15} genes. Plasmids of incompatibility type II, FIA, FIB, B/O, FIIS, and FIIA harboring *bla*_{CTX-M-15} gene have been reported in many *Salmonella* serovars [27, 62, 63]. Genes like *bla*_{SHV-12} and *mphA* found in *S. Worthington*–studied isolates were not

transferable, suggesting co-localization of the genes on chromosome or on non-conjugative plasmid. The role of plasmids in pansusceptible isolates needs further exploration.

MLST of the studied isolates revealed only one ST for serovars like *S. Worthington* (ST592), *S. Bareilly* (ST203), *S. Virchow* (ST16), *S. Weltevreden* (ST365), *S. Infantis* (ST32), and *S. Kentucky* (ST198), and two STs for serovars *S. Typhimurium* (ST36 and ST19) and *S. Enteritidis* (ST11 and ST1975). Phylogenetic clonal serovars by MLST as observed in this study was also reported in studies from other countries [29, 30, 61]. All STs reported in this study were the common STs generally found in the specific *Salmonella* serovars globally as evidenced in the MLST database (enterobase.warwick.ac.uk/species/senterica/search_strains). Isolation of Cip^R *S. Kentucky* ST198 MDR strains from children is alarming due to its association with the epidemic clone. The epidemic *S. Kentucky* ST198 clone, resistant to first-line antimicrobials and ciprofloxacin, originated in Egypt in 2002, and since then has spread rapidly through Africa, Middle-east, Asia, and Europe [23]. MLST showed good correlation between different *Salmonella* serovars and their STs may be used as an alternative to cumbersome serotyping for determining serovars, as was suggested by earlier researchers [29, 64]. However, MLST was less discriminatory than PFGE for epidemiological *Salmonella* surveillance. PFGE analysis could identify multiple pulsotypes among isolates having same STs (Fig. 4). No significant correlation among PFGE profiles, sequence types, AMR profiles, and plasmid profiles were found among the studied isolates. Although, genetically related strains (> 80% similarity coefficient) of each serovar were found in circulation, the presence of different serovar-specific PFGE clusters suggested the presence of multiple reservoirs of NTS in the environment and hence multiple sources for infection in children. An integrative study involving NTS isolated from humans, animals, and environmental samples would help to track the source of infection and subsequently prevent its spread among humans.

Most cases of NTS diarrhea remain unreported due to their self-limiting nature of infection and therefore this hospital-based surveillance study may not truly reflect the magnitude of *Salmonella* specific diarrheal disease in the community. However, the high level of resistance to 3GCs, FQs, and Azm observed in some serovars of NTS is a cause of concern and public health threat. Unnecessary antibiotic use for treatment of NTS gut infection should be avoided. The isolation of MDR isolates carrying transferable resistance genes on plasmids and integrons is worrisome and reinforces the importance of surveillance for monitoring *Salmonella* serovars and their AMR profiles to control the spread of resistant isolates. Prevention of infection in children by disseminating proper hand hygiene practice is also strongly recommended.

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Author contributions Conceptualization: Shanta Dutta. Methodology: Priyanka Jain, Sriparna Samajpati, Arindam Ganai, Surajit Basak. Data curation: Priyanka Jain, Goutam Chowdhury, Surajit Basak, Sandip Samanta. Formal analysis and investigation: Priyanka Jain, Surajit Basak, Asish K. Mukhopadhyay, Keinosuke Okamoto. Resources: Goutam Chowdhury, Sandip Samanta, Asish K. Mukhopadhyay, Keinosuke Okamoto. Supervision: Shanta Dutta. Writing-original draft preparation: Priyanka Jain, Shanta Dutta. Writing-review and editing: Goutam Chowdhury, Sriparna Samajpati, Surajit Basak, Arindam Ganai, Sandip Samanta, Keinosuke Okamoto, Asish K. Mukhopadhyay.

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Compliance with ethical standards

Conflict of interest The authors declare that there is no conflict of interest.

Ethical approval The present study was reviewed and approved (no. C-48/2011-T & E) by the Institutional Ethical Committee (IEC) of ICMR-National Institute of Cholera and Enteric Diseases (NICED), Kolkata.

Informed consent Informed consents were obtained from the parents or guardians of each patient enrolled in this study.

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