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Comparison of *Salmonella* genes in stool samples of children aged 5 years and younger in urban and rural areas of Bangladesh

방글라데시 도시와 시골 지역의 5살과 5살 미만 어린이의 대변에 있는 살모넬라 유전자 비교

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Comparison of *Salmonella* genes in stool samples of children aged 5 years and younger in urban and rural areas of Bangladesh

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Abstract

Introduction Typhoid incidence in children is higher in urban areas than in rural areas of Bangladesh. This study examined whether healthy urban children harbored more increased levels of *Salmonella* genes than healthy rural children.

Methodology Stool samples from 140 children were studied: 70 from rural areas and 70 from urban metropolitan areas.

DNA was extracted from 180 to 200 mg of stool samples according to the manufacturer ' s instructions using NucleoSpin Stool DNA extraction kit (Macherey-Nagel, Düren, Germany). We used nested PCR with a thermal cycler (Veriti 96-well thermal cycler, Applied Biosystems, Foster City, CA) to screen for the presence of *Salmonella* genes in healthy children' s stool specimens. Six *Salmonella* gene targets were used for amplification. *Salmonella* gene targets included 16S rRNA [75], *Salmonella* pathogenicity island I gene (*hilA*) [76], *Salmonella* enterotoxin gene (*stn*) [77], *invA* gene [78], Fur-regulated gene (*iroB*) [79], and histidine transport operon (*hisJ*) [80].

Results The stool samples of urban children contained more Salmonella genes (median 4, IQR 3−4) than rural children (median 3, IQR 3−4). This suggests that urban Bangladeshi children have more *Salmonella* genes in their guts than rural children. Especially in those under one year of age, the *Salmonella* gene prevalence in urban children was unique. They had more *Salmonella* genes (median 4, IQR 4−5) than rural children in the same age group (median 3, IQR 2.5−4). We also found more *Salmonella* genes in urban children who drank tap water (median 4, IQR 3−5) than in rural children whose water source was tube well water (median 3, IQR 2−4) and boiled pond water (median 3, IQR 3−3.5). However, there was no significant difference in *Salmonella* genes between urban children who drank tap water and children whose water source was a tube−well (median 4, IQR 3−4).

Conclusions These data suggest that the urban environment, including the drinking water supply system, increases the likelihood of healthy children in urban areas harboring more potentially pathogenic *Salmonella* organisms in their gut than

found in rural healthy children.

Keywords: *Salmonella* genes, Bangladesh, water supply system, urban children, rural children, typhoid.

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Introduction

1.1. Study Background

Typhoid fever is a critical health problem in many developing countries, including Bangladesh. An estimate for 2017 showed 14.3 million cases of typhoid and paratyphoid infections globally [1]. Also, nontyphoidal salmonellosis, related to gastroenteritis and sepsis, was estimated at 3.4 million cases in 2015 [2].

Bangladesh is one of the most densely populated countries globally, with an estimated population of 162.7 million and a population density of 1,103 persons per square kilometer in 2017 [3]. It is a riverine country and routinely faces disasters from monsoon floods and riverbank erosion that cause homelessness and landlessness and compel migration to cities searching for work. In one study, about 10% of the respondents migrated because of a natural disaster, and 70% of those migrants remained permanently in urban areas [4]. This in-migration creates huge infrastructure challenges for cities. Around 37% of the Bangladeshi population lived in urban areas in 2018 [5].

Consequently, urbanization in Bangladesh is typically chaotic.

The capital city-centric development strategy has led to an explosion of the population and geographic boundaries of the city of Dhaka without corresponding infrastructure expansion. As a result, basic urban utilities are now in acute shortage. Greenery and water bodies are disappearing, and slums adjoin high-rise buildings. Untreated sewage contaminates neighboring rivers [6].

Bangladesh, which lies between India and Myanmar, has a high incidence of salmonellosis and typhoid fever, which disproportionately affect younger children, with the highest incidence in children < 5 years old [3,7]. The incidence of invasive salmonellosis is high among residents of the densely populated urban communities in Dhaka and areas adjacent to the Dhaka metropolitan area [8]. This study reported 2.0 typhoid fever episodes (95% confidence interval [CI] 1.5-2.8)/1000 person-years in 2010 using a blood culture system. They also found that the incidence in children < 5 years old was 12-fold higher than the incidence among children ≥ 5 years of age.

In Bangladesh, the incidence of nontyphoidal salmonellosis has historically been higher in metropolitan areas than in rural villages. A study conducted in 1977-1979 found that of 214 isolates from blood and stool from metropolitan Dhaka, 0.3% of

66,341 cultures were positive compared with 12 (0.04%) of 27,265 positive cultures of isolates from rural areas [9–11]. A study of multidrug–resistant pathogenic bacteria in the gut of healthy young children in Bangladesh concluded that the gut of young children below the age of 5 years was an important reservoir for pathogenic bacteria [10].

Another study reviewed 19,265 blood cultures from an urban paediatric hospital in Dhaka. Of these, 855 (4.4%) were positive by culture for ST/PTF. The same study found 25 (0.2%) of 15,455 blood cultures from a rural hospital in Mirzapur were positive for *Salmonella* [11]. A 2005 community–based study in an urban slum in Bangladesh reported an overall incidence of salmonellosis of 3.9/1000 person–years and a higher rate in children aged 0–4 years (18.7/1000 person–years) [12]. Another study found a higher incidence in children aged < 5 years (10.5/1000 person–years) with an overall incidence rate of 2.0/1000 person–years [13].

Previous studies established that the pathogenesis of enteric fever depends on several factors, including the infecting species and infectious dose. When ingested in high doses, these organisms survive exposure to gastric acid before gaining access to the small bowel, where they penetrate the epithelium,

enter the lymphoid tissue, and disseminate via the lymphatic or hematogenous route [14–16].

1.2. Purpose of Research: Hypothesis

Several studies showed a higher incidence of typhoid fever in urban children than in rural children in Bangladesh. These studies attributed this higher incidence of typhoid fever in urban children to several factors, including high population density in slum areas, poor sanitary conditions, drinking unsafe water, and ingestion of contaminated food. No study, however, has examined whether the presence of *Salmonella* genes in the guts of children play a role in typhoid fever, i.e., whether the presence of higher numbers of *Salmonella* genes in the intestines of healthy urban children is a predisposing factor for a higher incidence of typhoid fever in urban children compared with rural children.

Most studies agree that both environment and infrastructure are related to a high incidence of typhoid fever in young urban Bangladeshi children. However, no studies have assessed whether the intestines of urban children harbor more *Salmonella*, even when they are healthy (i.e., not suffering from salmonellosis), than the guts of healthy rural children.

This study hypothesized that in Bangladesh, healthy urban children harbour more *Salmonella* genes in their gut as commensal bacteria than rural children. This reasoning was supported by previous studies [9,17], including one that showed children in urban areas were more likely to carry *Escherichia coli* [17].

To assess our hypothesis, we examined stool samples of healthy urban and rural children using molecular detection of *Salmonella* genes by two-step nested PCR to determine the mean number of *Salmonella* genes present in the intestines of the sample children.

Literature Review

2.1. Literature Review

Salmonella enterica subspecies *enterica* serovar Typhi (*Salmonella* Typhi, *S.* Typhi) is the cause of typhoid fever. Together, *S.* Typhi and *Salmonella* Paratyphi A (*S.* Paratyphi A) are the major agents of enteric fever. Like other typhoidal *Salmonella* serovars, *S.* Typhi is a human host-restricted organism. The role of water as a vehicle for typhoid fever has been appreciated since the late 1800s [18-19] and the role of food not long after [20]. Our understanding of the global burden of typhoid fever has improved in recent decades, with an increase in both the number and geographic representation of high-quality typhoid fever incidence studies and greater sophistication of modeling approaches.

The 2017 World Health Organization (WHO) Strategic Advisory Group of Experts on Immunization (SAGE) recommendation for the introduction of typhoid conjugate vaccines (TCVs) for infants and children aged >6 months in typhoid-endemic countries [21] is likely to require further improvements in our understanding of typhoid burden not only at the global level but also at the national and subnational levels. Furthermore,

recognizing the critical and synergistic role of water and sanitation improvements in concert with vaccine introduction [22] emphasizes the need to improve our understanding of the sources, patterns, and modes of transmission of *S.* Typhi in diverse local settings.

2.2. The organism

The bacterium is serological positive for lipopolysaccharide antigens O9 and O12, protein flagellar antigen Hd, and polysaccharide capsular antigen Vi. The Vi capsular antigen is largely restricted to *S.* Typhi, although it is shared by some strains of *S.* Paratyphi C and dublin, and Citrobacterfreundii [19]. Polysaccharide capsule Vi has a protective effect against the bactericidal action of the serum of an infected person [20].

Recent changes in enteric disease epidemiology have emerged in Africa, Asia, and Latin America [18, 21−23]. The people living in the slums of the developing world bear 21 million cases a year. Pakistan, India, and Bangladesh together account for 85% of the world cases [24]. Children and young adults had the highest age−specific rates of enteric infection. The mean age of those infected with typhoid fever is 7 years in Pakistan.

2.3. Epidemiology

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sources, patterns, and modes of transmission of *S.* Typhi in diverse local settings.

2.4. Chain of infection

2.4.1. Reservoir

Humans are the reservoir (defined as the habitat in which the agent lives typically, grows, and multiplies) of *S.* Typhi. *S.* Typhi has limited capacity to multiply outside of the human host. Still, it may survive for extended periods in the environment [25]. Acute *S.* Typhi infection presents as typhoid fever. Typhoid fever may be difficult to distinguish clinically from other febrile illnesses. If untreated, intestinal, neuropsychiatric, and other complications develop in some patients. However, acute infection can also be mild and self-limited. Human challenge studies demonstrate that fecal shedding and even bacteremia may occur without clinical signs of typhoid fever [26].

2.4.2. Portal of exit, route of infection, and source

Feces represent the major portal of exit of *S.* Typhi, although shedding in urine has also been documented [27]. *S.* Typhi may

be shed in the stool or urine during and following both clinical and subclinical acute infection. Shedding may be temporary or chronic. Temporary shedding may be acute or convalescent. A convalescent carrier sheds *S.* Typhi for ≥3–12 months after the onset of acute illness. A chronic carrier sheds typhoid bacillus for >12 months after the onset of acute illness. Practically speaking, a chronic carrier may be defined as someone with no history of typhoid fever or someone who had the disease >1 year previously, who has fecal or urine cultures positive for *S.* Typhi separated by at least 48 hours.

The relative contribution of temporary shedding versus shedding from chronic carriers to new infections remains an unanswered yet critical question for typhoid control and elimination. Chronic carriers are a major source of domestically acquired *S.* Typhi infections in countries with low typhoid incidence [28]. However, "Typhoid Mary" [29] has assumed a place in both popular and medical consciousness that belies the potentially greater contribution to transmission of temporary shedding in settings of high typhoid incidence. *S.* Typhi transmission is by the fecal-oral route. Water and food contaminated by human feces are the major sources (defined as the places from which the agent is transferred to a host) of *S.*

Typhi. The human reservoir is considered the source of *S.* Typhi occasionally, and ingestion of human feces during oral-anal sex has been implicated [29].

2.4.3. Mode and patterns of transmission

The mode of *S.* Typhi transmission is mainly indirect and predominantly vehicle-borne through contaminated water or food [30]. Water and food usually serve as passive vehicles for *S.* Typhi. While *S.* Typhi may survive for extended periods on vehicles, multiplication of *S.* Typhi in water and food is uncommon [23].

In short-cycle transmission, food and water are contaminated by fecal shedding in the immediate environment, and transmission is mediated through inadequate hygiene and sanitation measures. In long-cycle transmission there is the contamination of the broader environment, such as pollution of untreated water supplies by human feces and use of raw human feces or untreated sewage as a crop fertilizer [31].

Epidemiologic investigations underscore the important role of chronic carriers in short-cycle foodborne typhoid outbreaks in countries with low typhoid incidence [28], and the potentially

large scale of long-cycle waterborne transmission in many high-incidence settings [32]. The means by which the source is contaminated and the type of vehicles involved vary considerably from location to location, underscoring the importance of local epidemiologic investigations for informing nonvaccine control measures.

2.4.4. Portal of entry and host

The portal of entry for *S.* Typhi infection is the mouth, usually through ingestion of fecally contaminated water or food. Infection occurs in a susceptible human host. The incubation period shortens, and the risk for infection and disease increases with the ingested dose [33]. Gastric acid provides an important barrier to *S.* Typhi accessing the small intestinal mucosa and, in turn, the reticuloendothelial system. Natural and vaccine-induced immunity provide partial protection against typhoid fever [34].

2.5. Incidence

The incidence of a disease is the number of new cases per population per unit time. For typhoid fever, the incidence is usually expressed as cases per 100,000 population per year. Typhoid fever incidence is often classified as low, medium, high [35], and, more recently, very high [36], corresponding to incidence bands of <10, 10–100, >100–<500, and ≥500 per 100 000 per year, respectively. For the burden of disease calculations, population-based incidence measured by active disease surveillance accounting for blood culture insensitivity is used [35]. Studies combining sentinel healthcare facility surveillance with healthcare utilization surveys to account for under-ascertainment are increasingly done to approximate population-based disease incidence with lower resource investment [37-38].

Early attempts to estimate the global burden of typhoid fever were hampered by the very limited number of contemporary population-based studies of typhoid fever incidence using blood culture confirmation from typhoid-endemic areas [39-40]. Data from the control arm of the burgeoning number of typhoid vaccine trials were central to the 2,000 estimate of global typhoid incidence. Still, their use was tempered by concern for

bias due to the preference for conducting vaccine trials in high-incidence settings [35]. Since that time, several studies [41-43] have been completed that bolster data on typhoid fever incidence from an increasingly diverse range of locations. Such studies include those that are truly population-based, with household surveillance for fever and blood culture in the home or by active referral to healthcare facilities [44]. In addition, several recent incidence studies rely on healthcare facility-based surveillance supplemented with healthcare utilization surveys that provide multipliers to account for under-ascertainment related to healthcare access among febrile persons [45].

There is growing recognition of the considerable variation in typhoid fever incidence that may occur in place and over time. Whereas in 2000 typhoid fever appeared to be less common or under-ascertained in Africa than in Asia [35, 46], more recent studies confirm that typhoid fever incidence is high in some parts of Africa [44-45]. Furthermore, typhoid has been demonstrated to occur at high incidence after years of little disease in some locations [47] while declining markedly from high incidence levels elsewhere [45].

Furthermore, *Salmonella* serovars other than Typhi play differing roles by location. Among Asian bloodstream infection studies, both typhoid fever and paratyphoid fever are common [48]. African studies demonstrate that non-typhoidal *Salmonella* invasive disease is often as common or exceeds typhoid fever incidence in some locations [49]. Notwithstanding substantial improvements and changes in source data and methods for extrapolation, the annual number of typhoid fever illnesses has not kept pace with global population growth. However, typhoid fever still ranks high among the major causes of infectious disease illness and death [50-51].

2.6. Drivers of typhoid fever incidence

Perhaps not surprisingly, given predominant modes of *S.* Typhi transmission, early 20th century data from large cities in Europe and North America repeatedly demonstrated a reduction in typhoid fever illnesses and deaths ecologically associated with measures to improve the microbiologic quality of drinking water [52]. Similar health gains could likely be achieved in typhoid-endemic countries if human feces could reliably be excluded from drinking water and food. In 1990, sub-Saharan

Africa, South and Southeast Asia, and Oceania had the lowest population coverage with improved water and sanitation facilities. By 2015, water and sanitation coverage had increased markedly in South and Southeast Asia and less so in sub-Saharan Africa [53]. However, Oceania made little progress in either category during the same period and is now thought to experience the highest incidence of typhoid fever of any global region [54].

2.7. Disability and death

The case fatality risk of typhoid fever was approximately 10%-30% in the pre-antimicrobial era [55]. With effective antimicrobials, the case fatality risk is usually <1%. Antimicrobial-resistant *S.* Typhi is a major global health concern [56]. Clinical studies demonstrate the association between antimicrobial resistance and poor patient outcomes [57]. One of the most important modifiable contributors to a poor outcome in typhoid fever is a delay in instituting effective antimicrobial treatment. This delay is more likely to occur where typhoid fever is underrecognized as a cause of febrile illness [58]. Empiric antimicrobial therapy does not correspond

with patterns of antimicrobial susceptibility of local *S.* Typhi strains.

Population-based studies of typhoid fever incidence are rarely large enough to accurately estimate the prevalence of complications such as intestinal perforation and deaths. The early detection and enhanced clinical management of typhoid fever inherent and appropriate in such studies modify complications and deaths. Hospital-based studies are likely to be biased toward more severe disease or those with access to hospital-level healthcare [38]. To date, estimates of the proportion of typhoid fever patients developing complications and dying have been based on expert opinion and on systematic reviews of hospital-based studies that have inherent limitations. Research under way at present is anticipated to shore up estimates of typhoid fever complications and deaths.

2.8. Typhoid fever among infants and young children

A recent systematic review of pediatric enteric fever showed that typhoid fever is common among children <5 years of age in many locations [59]. However, the review also highlighted the lack of detailed data on typhoid occurrence in the youngest age groups, especially those <2 years of age. The figure placed

below illustrates potential drivers of typhoid fever incidence and uncertainty about typhoid fever incidence among infants and young children <2 years of age. Typhoid fever incidence likely describes a sigmoid curve in this age group, with low incidence in the neonatal period, rising with age.

A growing body of evidence demonstrates a substantial typhoid fever problem among infants and children in high typhoid fever incidence [47]. With TCVs able to protect infants and young children, WHO SAGE recently recommended the use of TCV among those aged >6 months in typhoid-endemic countries [60]. Data on typhoid fever occurrence among infants and young children and other age groups will be invaluable as countries strive to make evidence-based decisions about whether and at what age to introduce TCVs.

2.9. Clinical features

Typhoid fever is among the most common febrile illness encountered by physicians in developing countries. After an incubation period of 7 to 14 days, the onset of bacteremia is marked by fever and malaise. Patients typically present toward the end of the first week after the onset of symptoms with fever,

influenza-like symptoms with chills, headache, malaise, anorexia, nausea, poorly localized abdominal discomfort, dry cough, and myalgia. Coated tongue, tender abdomen, hepatomegaly, and splenomegaly are common [61-62].

The advent of antibiotic treatment has led to a change in the classic mode of presentation; a slow and stepladder rise in fever and toxicity is rarely seen nowadays. In areas where malaria is endemic and schistosomiasis is common, the presentation of typhoid may be atypical [63]. Even polyarthritis and monoarthritis are reported presentation [62].

Adults often have constipation, but in young children and adults with HIV infection, diarrhea is more common. The presentation of typhoid is more dramatic in children younger than 5 years, with higher rates of complications and hospitalization. Diarrhea, toxicity, and complications such as disseminated intravascular coagulation are also more common in infancy [62]. Typhoid fever during pregnancy, complicated by miscarriage, antimicrobial treatment has made this outcome less common. Vertical intrauterine transmission from an infected mother may lead to neonatal typhoid, a rare but severe and life-threatening condition [62].

Relapses occur in 5 to 10 percent of patients, usually two to

three weeks after the resolution of fever. The relapse is usually milder than the initial attack, and the *S.* Typhi isolate from a patient in relapse usually has the same antibiotic susceptibility pattern as the isolate obtained during the initial attack. Reinfection can only be distinguished from relapse by molecular typing [63–64].

2.10. Causes

- Contaminated food, often having no unusual look or smell.

- Poor kitchen hygiene, especially problematic in institutional kitchens and restaurants, can lead to a significant outbreak.
- Excretions from either sick or infected but apparently clinically healthy people and animals (especially dangerous are caregivers and animals).
- Polluted surface water and standing water (such as in shower hoses or unused water dispensers).
- Unhygienically thawed poultry (the meltwater contains many bacteria).
- An association with reptiles (pet tortoises, snakes, iguanas, and aquatic turtles) is well described.
- Amphibians such as frogs.

Salmonella bacteria can survive for some time without a host; they are frequently found in polluted water, with contamination from the excrement of carrier animals being particularly important. The European Food Safety Authority highly recommends that when handling raw turkey meat, consumers and people involved in the food supply chain should pay attention to personal and food hygiene.

2.11. Diagnosis

The diagnosis of typhoid can be made in the developing world from clinical criteria. In areas of endemic disease, fever without evident cause that lasts for more than one week should be considered typhoid until proven otherwise. It has distinguished itself from other endemic acute and subacute febrile illnesses. However, malaria, deep abscess, tuberculosis, amoebic liver abscesses, encephalitis, influenza, dengue, leptospirosis, infectious mononucleosis, brucellosis, rickettsial diseases, etc. should be considered [16]. 15% to 25% of patients show leucopenia and neutropenia. Leukocytosis is found in intestinal perforation and secondary infection [65]. In younger children, leukocytosis is the common association and may reach 20,000–

25,000/mm^3. Liver function tests may be deranged. Although significant hepatic dysfunction is rare, some studies and case reports showed there was hepatic derangement simulating acute viral hepatitis and was also present as a hepatic abscess.

Blood cultures are the standard diagnostic method; they are positive in 60% to 80% of patients with typhoid. The culture of the bone marrow is more sensitive, around 80% to 95% of patients, even in patients taking antibiotics for several days, regardless of the duration of illness. Blood culture is less sensitive than bone marrow because of fewer organisms in blood than bone marrow. The sensitivity of blood culture is higher in the first week of illness, increases with the volume of blood cultured (10–15 ml should be taken from schoolchildren and adults, 2–4 ml are required from toddlers and preschool children).

Toddlers have a higher level of bacteremia than an adult. Cultures have also been made from the buffy coat of blood, streptokinase treated blood clot, intestinal secretion (with the use of duodenal string capsule), and skin snips of rose spots. The sensitivity of stool culture depends on the amount of feces cultured, and the positivity rate increased with the duration of illness. Stool cultures are positive in 30% of patients with acute

typhoid fever [6, 7]. Urine culture have got 0–58% sensitivity [61].

2.12. Felix–Widal test

The classic Widal test is more than 100 years old. It detects agglutinating antibodies to the O and H antigens of *S.* Typhi. The levels are measured by using doubling dilutions of sera in a large test tube [66]. Although robust and simple to perform, this test has moderate sensitivity and specificity. Its reported sensitivity is 70% to 80%, with a specificity of 80% to 95%. It can be negative in up to 30 % of culture–proven typhoid fever because of blunted antibody response by earlier use of antibiotics. Moreover, patients with typhoid may mount no detectable antibody response or have no demonstrable rise in antibody titre. Unfortunately, *S.* Typhi shares these antigens with other *Salmonella* serotypes and shares these cross–reacting epitopes with other Enterobacteriaceae. This can lead to false positive results. Such results may also occur in other clinical conditions, e.g., malaria, typhus, bacteremia caused by different organisms, and cirrhosis.

In areas of endemicity, there is a low background level of

antibodies in the normal population. It is difficult to establish an appropriate cut-off point for a positive result since it varies between areas and between times in given areas. As a single acute serum is usually available, it is important to establish the antibody level in the normal population in a particular locality to determine a threshold above which the antibody titre is considered significant. A fourfold rise in the antibody titre between convalescent and acute sera is diagnostic if paired serums are available. Considering the low cost of the Widal test, it is likely to be the test of choice in many developing countries. This is acceptable, as long as the test results are interpreted with care, on the background of the previous history of typhoid, and in accordance to appropriate local cut-off values for the determination of positivity.

2.13. Evolution and usefulness of new diagnostic tools

Tubex test detects IgMO antibodies, *Typhi*dot detects IgM and IgG antibodies against 50 kD antigen of *S.* Typhi [67]. Tubex has not been evaluated extensively, but this test performed better than Widal test in both sensitivity and specificity in preliminary studies. Although culture remains the gold standard,

*Typhi*dot-M is superior to the culture method in sensitivity (93%) and has a high negative predictive value. In some studies, it has been shown that total Ig ELISA has superior sensitivity compared to other tests. Recently DNA probes and polymerase-chain-reaction (PCR) have been developed to detect *S.* Typhi directly in the blood. Urine antigen detection has 65-95% sensitivity. PCR still not been used in clinical practice.

2.14. Treatment

Early diagnosis and prompt institution of appropriate antimicrobial are essential for optimal management. Knowledge of antibiotic susceptibility is crucial in determining which drug to use. More than 90% of patients can be managed at home with oral antibiotics and regular follow-up. However, patients with severe illness, persistent vomiting, severe diarrhea, and abdominal distension, require hospitalization and parenteral antibiotic treatment. Chloramphenicol was the drug of choice for several decades after its introduction in 1948. However, the emergence of plasmid mediates resistance, and the development of serious side effects like bone marrow aplasia had pushed this

drug aside. Trimethoprim–sulfamethoxazole and ampicillin were employed to counter chloramphenicol resistance in 1970, but it was also discarded because of development of plasmid–mediated resistance [68].

In 1992, the emergence of multidrug resistance enteric fever (resistance to chloramphenicol, ampicillin, and trimethoprim–sulfamethoxazole) was strongly addressed in Bangladesh; around 36.58% of cases were reported in a large study [69]. In the 1980s, ceftriaxone and ciprofloxacin became the drug of choice [68]. Although fluoroquinolones attain excellent tissue penetration, rapid therapeutic response, and a very low rate of post–treatment carriage, strains of bacteria have emerged in Asia that show resistance to them in the past decade [68]. Resistance to fluoroquinolone may be total or partial. The nalidixic–acid–resistant strain has reduced susceptibility to fluoroquinolone drug compared to the nalidixic–acid–sensitive strain. Although isolates are nalidixic acid–resistant, these can be susceptible to fluoroquinolones in disc sensitivity testing. Disc sensitivity testing is defined as a ciprofloxacin MIC of 0.12–1 mg/L and is not always detected by testing nalidixic acid resistance [68]. The available fluoroquinolones (ofloxacin, ciprofloxacin, fleroxacin, perfloxacin) are highly active and

equivalent in efficacy. For nalidixic-acid-resistant infections, a minimum of seven days of treatment at the maximum permitted dosage is necessary, and 10-14 days are usually required.

Culture sensitivity data of the Dept. of Microbiology of BSMMU showed 8.6% sensitivity to nalidixic acid, whereas ciprofloxacin is still 67% sensitive. Nowadays, it is thought that gatifloxacin is better than older fluoroquinolones. The bacteria need dual point mutations (in the DNA-gyrase and Topoisomerase-4 genes) to become resistant to gatifloxacin. Most studies in endemic countries have identified gyrA mutation in *S. enterica* as a mechanism of resistance [70]. There is no reported pattern of sensitivity to gatifloxacin in Bangladesh. Azithromycin in a dose of 500 mg (10 mg/kg) given once daily for seven days has proven effective in the treatment of typhoid fever in adults and children. A dose of 1 g per day for five days was also found effective in adults. Of the third-generation cephalosporin, oral cefixime (15-20 mg per kg per day, for adults, 100-200 mg twice daily) has been widely used in children in various geographical settings and found to be satisfactory. However, in some trials, cefixime showed higher rates of failure and relapse than fluoroquinolones [68]. But antibiotic sensitivity pattern in BSMMU showed higher sensitivity, around 78.8%.

Intravenous third-generation cephalosporins (Ceftriaxone, Cefixime, Cefotaxime) are effective with low relapse (3 to 6%) and fecal carriage (< 3%) rates. Ceftriaxone is effective at a dose of 2-4 gm daily in a single or two divided doses. Aztreonam and imipenem are potential third-line drugs.

2.15. Antibiotic susceptibility pattern of isolated strains from the patients of three age groups (Farhana et al., 2015)

Antibiotics	Young children (n = 33)	Older children (n=23)	Adults (n= 16)
Resistance to ampicillin	13 (39)	6 (26)	2 (13)
Resistance to chloramphenicol	10 (30)	3 (13)	2 (13)
Resistance to co-trimoxazole	10 (30)	3 (13)	2 (13)
MDR*	5 (15)	3 (13)	2 (13)
Resistance to nalidixic Acid	33 (100)	23 (100)	14 (88) [a]
Reduced susceptibility to ciprofloxacin	33 (100)	23 (100)	14 (88) [a]
Resistance to ceftriaxone	0 (0)	0 (0)	0 (0)
Resistance to cefixime	0 (0)	0 (0)	0 (0)
Resistance to azithromycin	0 (0)	0 (0)	0 (0)

Results are n (%).

*MDR, multidrug-resistant (resistant to ampicillin, chloramphenicol and co-trimoxazole)

[a] $p < 0.05$ when compared to young children.

2.16. Prevention of typhoid

In Bangladesh, urban areas are rapidly growing compared to other parts of the world. In several studies, data indicate a higher infection rate in this urban population. Lack of safe water and inadequate sanitation is responsible for this increased incidence. In developing countries, reducing the number of

cases in the general population requires safe drinking water and effective sewage disposal. Food safety can be ensured by washing hands with soap before preparing food, water for drinking should be boiled, avoiding raw food shellfish, ice-cream.

In Dhaka city, people living close to the rivers Buriganga, Turag, and Balu had an elevated risk of typhoid. There are several factors responsible. Low-income inhabitants of this area frequently use surface water for drinking. As *S.* Typhi can survive in water for days, contaminated surface water acts as etiological agents of typhoid [71].

2.17. Vaccination

The first parental whole-cell typhoid vaccine was introduced in 1896 and used in England and Germany but withdrawn by most countries because of strong side effects [72]. The live oral vaccine Ty21a is available in enteric-coated capsules or liquid formulation. It should be taken in three doses two days apart on an empty stomach and suitable for adults and children at least 5 years. The vaccine is well tolerated. Because it is live attenuated, it should not be given immunocompromised and

patients taking antibiotics [73].

The parenteral Vi-based vaccine is suitable for adults and children over the age of two years. A single dose of 0.5 ml is administered intramuscularly. In field trials conducted in Nepal and South Africa, where Typhoid is endemic, the vaccine's protective efficacy was 72%, one and half years after vaccination. A booster dose is recommended every two years.

WHO recommends vaccination for people travelling in high-risk areas, where the disease is endemic for people in refugee camps, microbiologists, sewage workers, and children.

2.18. Future vaccine

A new Vi conjugate vaccine bound to nontoxic recombinant *Pseudomonas aeruginosa* has enhanced immunogenicity in adults and children aged 5-14 years. It is recently evaluated in Vietnam, and the efficacy of the vaccine in children aged 2-5 years after 27 months was found 91.2%. An important advantage of this vaccine is that it has the potential to be immunogenic in infants under the age of two [52]. Currently, the DNA vaccine of typhoid fever is in phase 1 and 2 clinical trials [72].

Methodology

3.1.1. Place of Study

This study has been conducted jointly in Wide River Institute of Immunology, Department of Biomedical Science, Seoul National University, South Korea, and Department of Microbiology, Bangladesh University of Health Sciences Hospital, Dhaka.

3.1.2. Period of study

The study was carried out from April 2016 to June 2018.

3.1.3. Type of study

The study was designed as a cross-sectional one.

3.1.4. Sample size

140 (70 from rural and 70 from urban children).

3.1.5. Study population

A total of 140 healthy children, 70 from the rural area of Sirajganj upazilla (subdistrict) and 70 from the metropolitan area of Narayanganj city, Dhaka.

3.1.6. Inclusion criteria

Healthy children from 0 to 5 years old.

3.1.7. Exclusion criteria

I. Children above 5 years old.

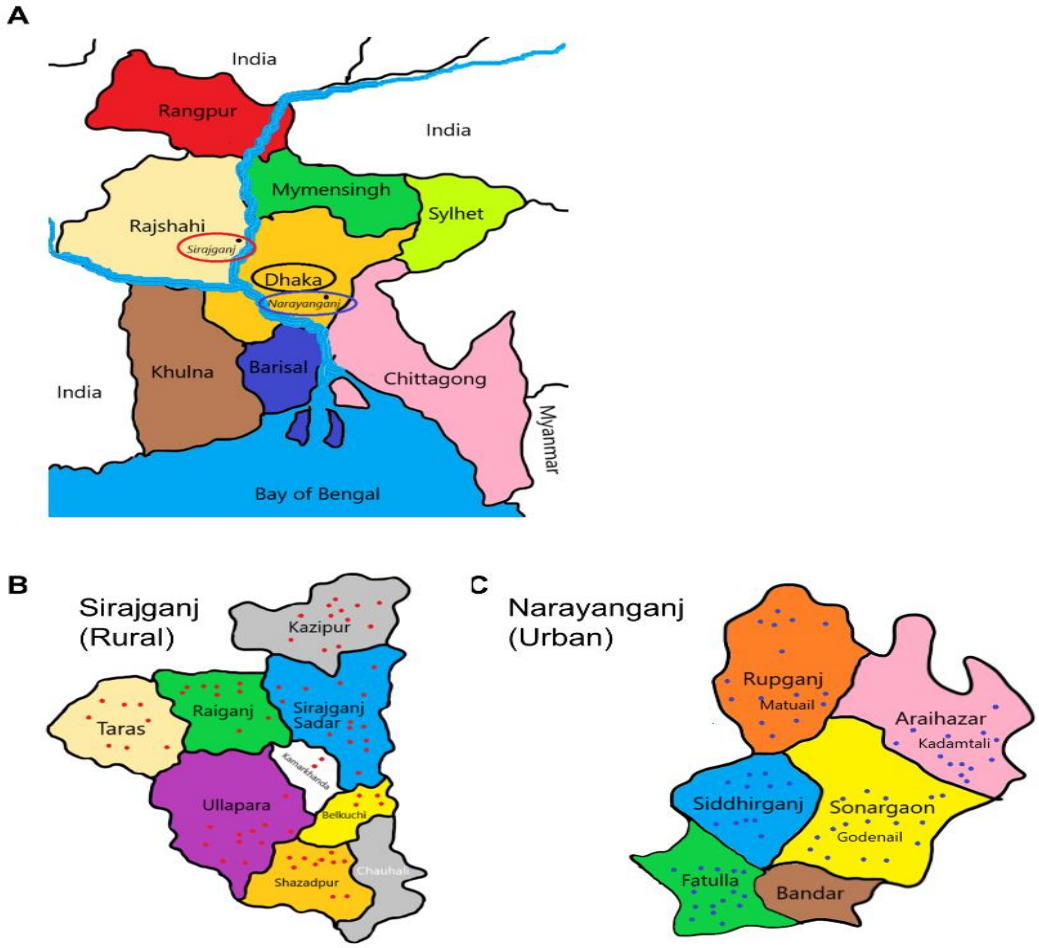
II. Children having diarrhea, vomiting, fever.

III. History of having antibiotics within the past six months.

3.2. Stool sample collection

In accord with this study's objective, we collected samples in two settings: the urban district of Narayanganj and the rural district of Sirajganj. The 2011 population densities of the two districts were 4,308 and 1,290 per square kilometre, respectively [74]. To ensure representative sampling, we collected samples in five separate administrative areas within Narayanganj (Fatulla, Godenail, Kadamtoli, Matuail, and Siddhirganj) and eight areas of Sirajganj (Belkutchi, Kamarkhand, Kazipur, Rayganj, Sadar, Shahjadpur, Tarash, and Ullapara). Narayanganj is adjacent to Dhaka, a metropolitan city, and has similar sanitary and water-supply infrastructure and overcrowding. The study enrolled 140 healthy children aged < 5 years (70 urban and 70 rural). The household selection was random, and samples were accepted only from children who could fulfill all study criteria. We also collected information from each child's parents on the child's eating habits, personal hygiene practices, and the source of the family's drinking water (Figure 1).

Figure 1 Sample Collection Areas



(A) Sirajganj (a rural district in northern Bangladesh, composed mostly of villages; red circle) and Narayanganj (blue circle, an urban city adjacent to the capital city of Dhaka, black circle). (B) Rural sample collection points in Sirajganj district (70 red dots show collection points for 70 stool samples in eight different areas). (C) Urban sample collection points in Narayanganj metropolitan city corporation (70 blue dots where 70 stool samples were collected from five different areas).

We began by collecting samples in the rural district of Sirajganj (in May 2016), followed by sample collection in the urban district of Narayanganj (in June 2016). During sample collection, healthy children lived at home and did not have any disease/symptoms of gastroenteritis (e.g., vomiting, diarrhoea, fever) or a history of taking antibiotics during the previous 6

months.

Health workers visited the children at home, obtained informed and written consent from their parents, and collected demographic information such as age, sex, personal hygiene, and dietary habits the day before getting samples. The parents collected the samples at home and put them in a refrigerator or the supplied icebox (if the household lacked refrigeration) at 4° C. The next day, the health workers collected stool samples in the containers provided to the parents, placed them in an icebox, and immediately transported the samples to a laboratory where samples were stored at −20℃ until processing for DNA extraction.

3.3. DNA extraction

DNA was extracted from 180 to 200 mg of stool samples according to the manufacturer ' s instructions using the NucleoSpin Stool DNA extraction kit (Macherey−Nagel, Düren, Germany). The amount of eluted DNA was measured by Nanodrop 2000 Spectrophotometer (Thermo Fisher Scientific, Waltham, MA) and stored at −20℃. The DNA integrity was estimated using 2% (w/v) agarose gel electrophoresis in a 0.5X TAE buffer. We mixed 1 μL of Midori Green with 10 μL of

extracted DNA, then loaded it on the gel. These DNA samples were used for PCR.

3.4. Molecular detection by nested PCR

We used nested PCR with a thermal cycler (Veriti 96-well thermal cycler, Applied Biosystems, Foster City, CA) to screen for the presence of Salmonella genes in healthy children's stool specimens. In primary PCR, six *Salmonella* gene targets were used for amplification (Table 1).

Table 1 Primary PCR primers used for screening of *Salmonella* genes

Target fragment	Primer (5' - 3')
16S rDNA	F: TGT TGT GGT TAA TAA CCG CA R: CAC AAA TCC ATC TCT GGA
iroB	F: TGC GTA TTC TGT TTG TCG GTC C R: TAC GTT CCC ACC ATT CTT CCC
hilA	F: CTG CCG CAG TGT TAA GGA TA R: CTG TCG CCT TAA TCG CAT GT
hisJ	F: ACT GGC GTT ATC CCT TTC TCT GGT G R: ATG TTG TCC TGC CCC TGG TAA GAG A
invA	F: GCT GCG CGC GAA CGG CGA AG R: TCC CGG CAG AGT TCC CAT T
Stn	F: CTT TGG TCG TAA AAT AAG GCG R: TGC CCA AAG CAG AGA GAT TC

All primers underwent 35 cycles.

Salmonella gene targets included 16S rRNA [75], *Salmonella* pathogenicity island I gene (*hilA*) [76], *Salmonella* enterotoxin gene (*stn*) [77], *invA* gene [78], Fur-regulated gene (*iroB*) [79], and histidine transport operon (*hisJ*) [80]. For primary PCR, we used TopSimple nTaq premix (Enzymomics, Daejeon, Korea) for amplification. The total reaction volume was 20 μL

(10 μL of pre-mix and 10 μL of master mix) (forward primer, 1 μL; reverse primer, 1 μL; stool DNA, 1 μL [10 ng/μL]; and ddH_2O, 7 μL). All primers were amplified for 35 cycles (Table 1). For secondary PCR, 1 μL of product from primary PCR was used to amplify the target band with different sets of primers (Table 2).

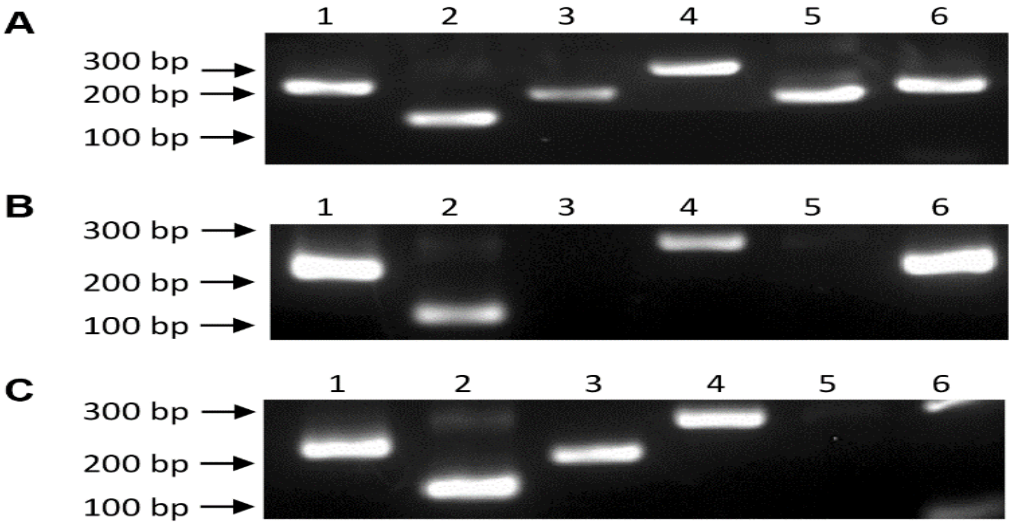
Table 2 Secondary PCR primers used for screening of *Salmonella* genes

Target fragment	Primer (5' -3')
16s rDNA	F: TTA CCC GCA GAA GAA GCA CC R: GCA TTT CAC CGC TAC ACC TG
iroB	F: TCA GCG AAG AGA TGA CCG AC R: GGC GGT AGG CGT TAG AAA GT
hilA	F: GAA CAC CAA CCC GCT TCT CT R: AAA ATC CCC ATT TGC GCC AT
hisJ	F: TGC TCA TTG CCG AAG GTC TC R: GGA TGC GCT GAT TCC GTC TT
invA	F: TCC TTT GAC GGT GCG ATG AA R: ATC GCA ATC AAC AAT GCG GG
Stn	F: GCG TAA AAA TCG CCT CCA GC R: CTA TTC ATG CGA TTG GCC GC

All primers underwent annealing at 58° C for 30 cycles.

PCR conditions were as follows: initial denaturation at 95℃ for 5 minutes, denaturation at 95℃ for 30 seconds, annealing at 58℃ for 30seconds, extension at 72℃ for 45seconds, and final extension at 72℃ for 10 minutes. Amplification products and sizes were determined by electrophoresis using 2% agarose gels. PCR amplification was considered positive if the amplicon matched the anticipated size and negative if no amplicon was detected (Figure 2).

Figure 2 Representative amplification bands of *Salmonella* genes



PCR amplification products were loaded onto 2% agarose gel and electrophoresis was performed for band separation. Lane 1, 16s rRNA; lane 2, Stn; lane 3, iroB; lane 4, hilA; lane 5, invA; lane 6, hisJ. (A) Samples from typhoid cases. (B) Representative samples from healthy rural children. (C) Representative samples from healthy urban children

Salmonella gene counts were scored according to the number of positive *Salmonella* gene bands shown in the nested PCR using multiple replications of single stool DNA samples and each of the *Salmonella* gene primer pairs. The primer set used for 16s rRNA detection failed to amplify genes of some *Salmonella* organisms but was also negative for many genetically related intestinal bacteria (e.g., *Citrobacter*, *Klebsiella*, *Proteus*, and *Escherichia* species). Some genes, including *hisJ*, *iroB,* and *invA* are well-conserved in non-*Salmonella* species but are not detected by *Salmonella*-specific primers. Primer pairs for other factors were not tested for non-Salmonella species in other studies [81].

3.5. Statistical analysis

We used the Shapiro–Wilk test to determine whether *Salmonella* gene values were normally distributed. Multivariable logistic regression analysis was used for comparing the effects of multiple factors to the binary–categorized Salmonella gene number (detected genes ≤ 3 or detected genes ≥ 4) in rural and urban children (SPSS software version 12.0; SPSS, Chicago, IL, USA). Statistical significance for continuous variables among and between groups was assessed by the Kruskal–Wallis test. The Chi–square test or Fisher's exact test was used to compare the proportion of each factor in rural and urban areas (GraphPad Prism software for Windows, version 5.01). Differences were considered significant at $p < 0.05$.

3.6. Ethics statement

I carried out this study in South Korea and in Bangladesh during the period from January 2016 to May 2018. The Department of Biomedical Sciences, Seoul National University (SNU) College of Medicine, Republic of Korea, and the Department of Microbiology and Immunology, Bangladesh University of Health Sciences (BUHS), Dhaka, supported me in conducting the study.

Because the children of this study were minors, we obtained written informed consent from their parents, who voluntarily agreed to participate in the study after the study details were explained. All personal information has been kept confidential – as per ethics requirements. This study was approved by the ethical review committee of BUHS, Dhaka.

Result

4.1. Salmonellosis in urban and rural children

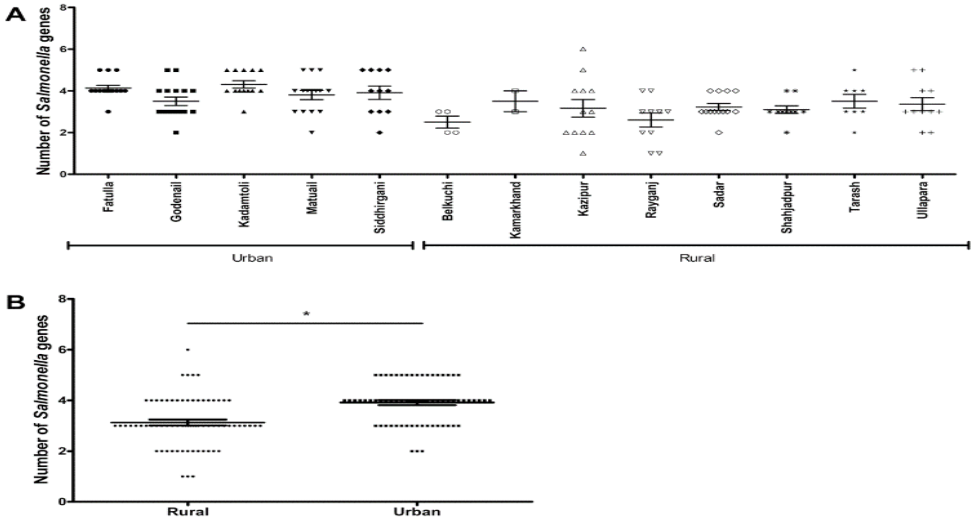
We first tested five stool DNA samples from *Salmonella*–culture–positive patients, and all samples produced the expected band sizes (16S rRNA, 215 bp; iroB, 201 bp; hilA, 281 bp; hisJ, 231 bp; invA, 197 bp; Stn, 132 bp) for every *Salmonella* gene assessed by nested PCR (Figure 2A). Of the 140 stool samples analysed, seven (3 from rural areas [Sirajganj] and 4 from urban areas [Narayanganj]) were negative for 16s rRNA. We then used *Salmonella* gene counts (0–6) as determined by positive *Salmonella* gene bands in nested PCR to score all samples (Table 3).

Table 3 Multivariate analysis of *Salmonella* gene based on associated factors

Factors		Crude OR	95% CI	Adjusted OR	95% CI
Area	Rural	1	(ref)	1	(ref)
	Urban	4.792	(2.35–9.81)	3.328	(0.9–12.32)
Hygiene	Modern WC, wash	6.133	(2.38–15.87)	1.518	(0.29–8.18)
	Flush/pit latrine, wash	1.971	(0.79–4.96)	0.537	(0.07–4.1)
	Pit latrine w/o slab, w/o wash	1	(ref)	1	(ref)
Age	0–12 months	1.467	(0.55–3.95)	NC	
	13–24 months	0.833	(0.31–2.29)	NC	
	25–36 months	2.375	(0.79–7.21)	1.618	(0.49–5.44)
	37–48 months	0.364	(0.1–1.43)	0.357	(0.08–1.61)
	49–60 months	1	(ref)	1	(ref)
Dietary habit	Breast milk	1	(ref)	1	(ref)
	Normal food	1.2	(0.62–2.34)	> 999.999	NA
Water supply	Pond water	1	(ref)	1	(ref)
	Tap water	6.815	(2.58–18.03)	0	(0–0)
	Tube well	2.282	(0.9–5.85)	2.524	(0.33–19.8)
Gender	Boy	1	(ref)	1	(ref)
	Girl	1.007	(0.52–1.96)	1.248	(0.59–2.68)

OR: odds ratio, CI: confidence interval, NC: not calculated. Adjusted OR for Salmonella was calculated from the multivariable logistic model, including area, hygiene, dietary habit, water supply, age, and gender simultaneously. Pit latrine w/o slab, w/o wash indicates children use a pit latrine without a slab and do not wash hands afterward; flush/pit latrine, wash indicates children use a flush or a pit latrine and wash their hands after visiting latrine and before eating food; modern WC, wash indicates children use the modern toilet, wash hands after defecation and before eating food.

Figure 3 Children in an urban Bangladesh region have more Salmonella genes than children in a rural area



(A) Number of Salmonella genes detected by nested PCR in samples by collection site. (B) Number of Salmonella genes grouped by origin (rural and urban areas). Rural (o), urban (Δ). Date were grouped and analysed by Mann–Whitney test. ***p<0.001.

We found significantly more amplified *Salmonella* genes in urban samples than in rural samples (Figure 3A and Figure 3B). In this study, the stool samples of urban children contained more *Salmonella* genes (mean, 3.92 per sample) than found in rural children (mean, 3.13 per sample).

4.2. Drinking-water source and salmonellosis in children

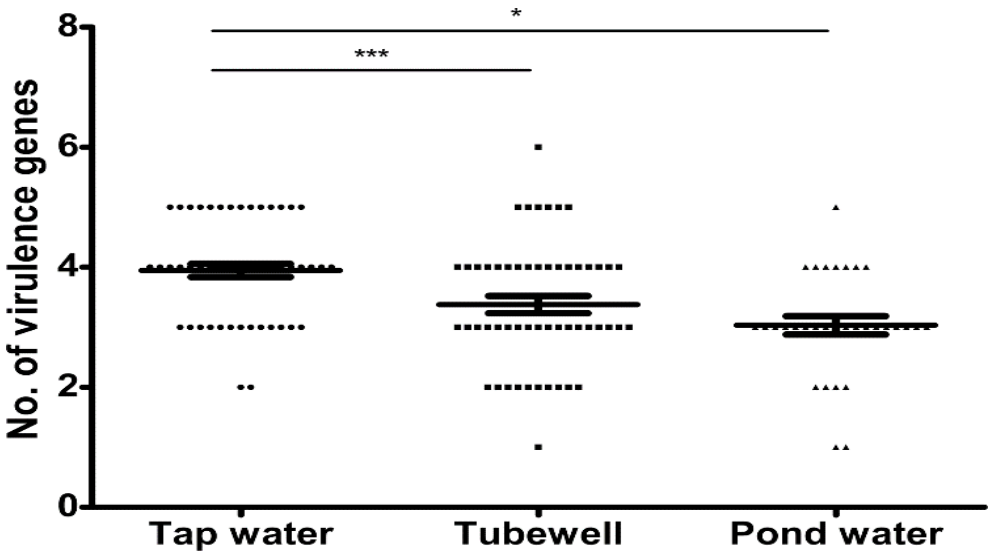
As water is an important medium of salmonellosis transmission, we examined the findings for each drinking-water source. Of the rural children studied, 38 (54.3%) drank water from tube wells, and 32 (45.7%) consumed boiled pond water. Among the urban children, 55 (78.6%) consumed tap water and 15 (21.4%) water from tube wells (Table 4).

Table 4 Age, dietary habit, water source, and hygiene of children studied

		Participant Number (n = 70, each group)			Average Gene Number		
		Urban	Rural	*P*	Urban	Rural	*P*
Age	0–12 months	21	16	0.19	4.29	2.94	< 0.01
	13–24 months	15	18		3.67	3.22	
	25–36 months	17	10		3.94	3.50	
	37–48 months	4	11		3.50	2.64	
	49–60 months	13	15		3.69	3.33	
Dietary habit	Breast milk	5	3	0.74 (F)	4.60	3.67	< 0.01
	Breast milk and soft food	31	34		3.94	3.00	
	Normal food	34	33		3.79	3.21	
Water source	Tube well	15	38	< 0.01	3.8	3.21	< 0.01
	Tap water	55	0		3.95	–	
	Boiled pond water	0	32		–	3.03	
Hygiene	Modern WC, wash	55	0	< 0.01	3.95	–	< 0.01
	Flush toilet/pit latrine, wash	15	37		3.8	3.30	
	Pit latrine w/o slab, w/o wash	0	33		–	2.94	

Frequency of age, diet, water source, and hygiene between urban and rural children were compared using chi-square or Fisher's exact tests. Likewise, gene number distributions between urban and rural children were assessed by Kruskal-Wallis test. WC, water closet; w/o, without; wash, handwashing.

Figure 4 Urban children whose tap water harbours more *Salmonella* genes



Number of Salmonella genes was compared in groups with different drinking water sources using Kruskal–Wallis test. Rural (◯), urban (◿). **p < 0.01, ***p < 0.001.

Children who drank tap water had the highest number of *Salmonella* genes (mean, 3.95 per sample), followed by children who drank water from tube wells (urban and rural tube wells, respectively, mean 3.80 and 3.24 per sample) and boiled pond water (mean 3.03 per sample) (Figure 4).

These differences suggest that water source may affect the prevalence of *Salmonella* genes. However, in the multivariable analysis, water supply system did not significantly affect the *Salmonella* gene number when other factors were adjusted constantly (Table 5).

Table 5 Data for each child in study and detection results of *Salmonella* genes from stool samples by nested PCR

Area	Sample No.	Hygiene	Age (months)	Dietary habit	Water supply	Address (city)	Gender	16s	*iroB*	*Stn*	*hilA*	*invA*	*hisJ*	No. of genes	Category
Rural	1	Flush/pit latrine, wash	0–12	Breast milk and soft food	Tube well	Sadar	Girl	+	+	+	+	−	−	4	1
Rural	2	Flush/pit latrine, wash	49–60	Normal food	Tube well	Sadar	Boy	+	+	−	+	−	−	3	0
Rural	3	Flush/pit latrine, wash	0–12	Breast milk and soft food	Pond water	Rayganj	Boy	+	−	−	+	+	−	3	0
Rural	4	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Rayganj	Girl	+	−	+	+	+	−	4	1
Rural	5	Flush/pit latrine, wash	25–36	Normal food	Tube well	Sadar	Boy	+	−	−	+	−	+	3	0
Rural	6	Pit latrine w/o slab, w/o wash	0–12	Breast milk	Pond water	Rayganj	Girl	+	−	−	+	+	−	3	0
Rural	7	Pit latrine w/o slab, w/o wash	37–48	Normal food	Pond water	Rayganj	Girl	+	−	−	+	−	−	2	0
Rural	8	Flush/pit latrine, wash	0–12	Breast milk	Tube well	Kazipur	Boy	+	−	−	+	−	+	3	0
Rural	9	Flush/pit latrine, wash	0–12	Breast milk	Tube well	Ullapara	Girl	+	+	+	+	+	−	5	1
Rural	10	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Tarash	Girl	+	+	+	+	−	−	4	1
Rural	11	Pit latrine w/o slab, w/o wash	37–48	Normal food	Pond water	Rayganj	Boy	+	+	−	+	−	−	3	0
Rural	12	Pit latrine w/o slab, w/o wash	37–48	Normal food	Pond water	Rayganj	Girl	+	−	+	+	−	−	3	0
Rural	13	Flush/pit latrine, wash	25–36	Normal food	Tube well	Sadar	Girl	+	−	+	+	+	−	4	1
Rural	14	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	+	+	+	−	−	4	1
Rural	15	Flush/pit latrine, wash	0–12	Breast milk and soft food	Tube well	Kazipur	Boy	+	−	+	+	−	−	3	0
Rural	16	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Belkuchi	Girl	+	−	−	+	−	−	2	0
Rural	17	Flush/pit latrine, wash	37–48	Normal food	Tube well	Sadar	Girl	+	−	+	+	−	−	3	0
Rural	18	Flush/pit latrine, wash	37–48	Normal food	Tube well	Sadar	Boy	+	−	−	+	−	−	2	0
Rural	19	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Sadar	Boy	+	−	+	+	−	−	3	0
Rural	20	Pit latrine w/o slab, w/o wash	25–36	Normal food	Pond water	Belkuchi	Girl	+	+	−	+	−	−	3	0
Rural	21	Pit latrine w/o slab, w/o wash	49–60	Normal food	Pond water	Belkuchi	Boy	+	−	+	+	−	−	3	0
Rural	22	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Sadar	Boy	+	−	+	+	−	−	3	0
Rural	23	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Sadar	Girl	+	+	−	+	−	−	3	0
Rural	24	Flush/pit latrine, wash	25–36	Normal food	Tube well	Ullapara	Boy	+	−	−	+	−	−	2	0
Rural	25	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	+	+	−	−	3	0
Rural	26	Flush/pit latrine, wash	49–60	Normal food	Tube well	Tarash	Boy	+	+	+	+	−	−	4	1
Rural	27	Flush/pit latrine, wash	49–60	Normal food	Tube well	Tarash	Girl	+	−	+	+	+	−	4	1
Rural	28	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Kamarkhand	Girl	+	−	+	+	+	−	4	1
Rural	29	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Kamarkhand	Boy	+	−	+	+	−	−	3	0
Rural	30	Pit latrine w/o slab, w/o wash	25–36	Normal food	Pond water	Tarash	Boy	+	+	+	+	+	−	5	1
Rural	31	Pit latrine w/o slab, w/o wash	37–48	Normal food	Pond water	Tarash	Boy	+	−	+	−	+	−	3	1
Rural	32	Pit latrine w/o slab, w/o wash	25–36	Normal food	Tube well	Tarash	Girl	−	−	+	+	−	−	2	1
Rural	33	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Ullapara	Girl	+	−	+	+	+	−	4	1
Rural	34	Flush/pit latrine, wash	49–60	Normal food	Tube well	Sadar	Girl	+	−	+	+	−	−	3	0

Rural	35	Flush/pit latrine, wash	49–60	Normal food	Tube well	Sadar	Girl	+	−	+	+	−	+	4	1
Rural	36	Flush/pit latrine, wash	49–60	Normal food	Tube well	Sadar	Boy	+	−	+	+	+	−	4	1
Rural	37	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Sadar	Girl	+	−	−	+	+	−	3	0
Rural	38	Flush/pit latrine, wash	49–60	Normal food	Tube well	Ullapara	Girl	+	−	+	−	+	−	3	0
Rural	39	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Tarash	Girl	+	−	+	−	+	−	3	0
Rural	40	Flush/pit latrine, wash	49–60	Normal food	Tube well	Ullapara	Boy	+	−	+	+	+	−	4	1
Rural	41	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	−	+	−	−	2	0
Rural	42	Flush/pit latrine, wash	49–60	Normal food	Tube well	Ullapara	Boy	+	−	+	+	−	−	3	0
Rural	43	Pit latrine w/o slab, w/o wash	37–48	Normal food	Pond water	Rayganj	Boy	+	+	+	+	−	−	4	1
Rural	44	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	+	+	−	−	3	0
Rural	45	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	−	+	+	+	4	1
Rural	46	Pit latrine w/o slab, w/o wash	37–48	Normal food	Tube well	Rayganj	Girl	−	−	+	+	−	−	2	0
Rural	47	Flush/pit latrine, wash	25–36	Normal food	Tube well	Ullapara	Boy	+	−	+	+	−	−	3	0
Rural	48	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Ullapara	Boy	+	+	+	+	+	−	5	1
Rural	49	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Ullapara	Boy	+	−	+	+	−	−	3	0
Rural	50	Pit latrine w/o slab, w/o wash	49–60	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	+	+	−	−	3	0
Rural	51	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Ullapara	Girl	+	−	−	+	−	+	3	0
Rural	52	Flush/pit latrine, wash	49–60	Breast milk and soft food	Tube well	Ullapara	Boy	+	−	−	+	−	−	2	0
Rural	53	Flush/pit latrine, wash	49–60	Normal food	Tube well	Kazipur	Girl	+	−	+	+	+	−	4	1
Rural	54	Pit latrine w/o slab, w/o wash	49–60	Normal food	Pond water	Shahjadpur	Girl	+	−	+	+	−	−	3	0
Rural	55	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	+	+	−	−	3	0
Rural	56	Pit latrine w/o slab, w/o wash	49–60	Breast milk and soft food	Pond water	Shahjadpur	Boy	+	−	+	+	−	−	3	0
Rural	57	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Rayganj	Boy	+	−	−	−	−	−	1	0
Rural	58	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Rayganj	Girl	+	−	−	−	−	−	1	0
Rural	59	Flush/pit latrine, wash	0–12	Breast milk and soft food	Tube well	Kazipur	Girl	+	−	−	−	−	−	1	0
Rural	60	Pit latrine w/o slab, w/o wash	13–24	Breast milk and soft food	Tube well	Kazipur	Boy	+	−	−	+	−	−	2	0
Rural	61	Flush/pit latrine, wash	0–12	Breast milk and soft food	Pond water	Shahjadpur	Girl	+	−	−	+	−	+	3	0
Rural	62	Flush/pit latrine, wash	37–48	Normal food	Tube well	Kazipur	Boy	+	−	−	+	−	−	2	0
Rural	63	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Kazipur	Boy	+	−	−	+	−	−	2	0
Rural	64	Flush/pit latrine, wash	37–48	Normal food	Tube well	Kazipur	Boy	+	−	−	+	−	−	2	0
Rural	65	Pit latrine w/o slab, w/o wash	0–12	Breast milk and soft food	Pond water	Belkuchi	Boy	+	−	−	+	−	−	2	0
Rural	66	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Kazipur	Girl	+	+	+	+	+	+	6	1
Rural	67	Flush/pit latrine, wash	25–36	Normal food	Tube well	Kazipur	Girl	−	+	+	−	+	+	4	1
Rural	68	Pit latrine w/o slab, w/o wash	37–48	Normal food	Pond water	Tarash	Boy	+	+	+	−	−	−	3	0
Rural	69	Flush/pit latrine, wash	25–36	Normal food	Tube well	Kazipur	Boy	+	+	+	+	+	−	5	1
Rural	70	Flush/pit latrine, wash	25–36	Normal food	Tube well	Kazipur	Girl	+	+	+	−	+	−	4	1
Urban	1	Modern WC, wash	25–36	Normal food	Tap water	Siddhirganj	Girl	+	+	+	+	+	−	5	1

Urban	2	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Siddhirganj	Boy	+	+	+	+	−	+	5	1
Urban	3	Modern WC, wash	25–36	Normal food	Tap water	Siddhirganj	Girl	+	−	+	+	−	−	3	0
Urban	4	Modern WC, wash	25–36	Normal food	Tap water	Siddhirganj	Girl	+	+	+	+	−	−	4	1
Urban	5	Modern WC, wash	49–60	Normal food	Tap water	Siddhirganj	Boy	−	+	+	+	+	−	4	1
Urban	6	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Siddhirganj	Girl	+	−	+	−	−	−	2	0
Urban	7	Modern WC, wash	0–12	Breast milk	Tap water	Siddhirganj	Girl	+	+	+	−	−	−	3	0
Urban	8	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Siddhirganj	Boy	+	+	+	+	+	−	5	1
Urban	9	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Siddhirganj	Girl	+	+	+	−	+	−	4	1
Urban	10	Flush/pit latrine, wash	25–36	Normal food	Tube well	Matuail	Boy	+	+	+	−	+	−	4	1
Urban	11	Flush/pit latrine, wash	0–12	Breast milk	Tube well	Matuail	Girl	+	+	+	+	+	−	5	1
Urban	12	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Matuail	Boy	+	+	+	+	+	−	5	1
Urban	13	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Matuail	Girl	+	+	+	−	+	−	4	1
Urban	14	Flush/pit latrine, wash	49–60	Normal food	Tube well	Matuail	Boy	+	+	+	−	+	−	4	1
Urban	15	Flush/pit latrine, wash	25–36	Normal food	Tube well	Matuail	Boy	+	+	+	−	+	+	5	1
Urban	16	Flush/pit latrine, wash	49–60	Normal food	Tube well	Matuail	Boy	+	−	+	−	+	−	3	0
Urban	17	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Matuail	Boy	+	−	+	+	+	−	4	1
Urban	18	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Matuail	Boy	+	+	+	−	+	−	4	1
Urban	19	Flush/pit latrine, wash	13–24	Breast milk and soft food	Tube well	Matuail	Boy	+	+	+	−	+	−	4	1
Urban	20	Flush/pit latrine, wash	0–12	Breast milk and soft food	Tube well	Matuail	Girl	+	−	+	−	+	−	3	0
Urban	21	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Kadamtoli	Boy	+	−	+	+	+	−	4	1
Urban	22	Modern WC, wash	0–12	Breast milk	Tap water	Kadamtoli	Girl	+	−	+	+	+	+	5	1
Urban	23	Modern WC, wash	0–12	Breast milk	Tap water	Kadamtoli	Boy	+	−	+	+	+	+	5	1
Urban	24	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Kadamtoli	Girl	+	−	+	+	+	+	5	1
Urban	25	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Kadamtoli	Boy	+	−	+	+	+	−	4	1
Urban	26	Modern WC, wash	37–48	Normal food	Tap water	Kadamtoli	Boy	+	+	+	−	+	−	4	1
Urban	27	Modern WC, wash	25–36	Normal food	Tap water	Kadamtoli	Boy	+	+	+	−	+	+	5	1
Urban	28	Modern WC, wash	25–36	Normal food	Tap water	Kadamtoli	Boy	+	−	−	+	+	+	4	1
Urban	29	Modern WC, wash	25–36	Normal food	Tap water	Kadamtoli	Boy	+	+	−	+	+	−	4	1
Urban	30	Modern WC, wash	0–12	Breast milk	Tap water	Kadamtoli	Boy	+	+	−	+	+	+	5	1
Urban	31	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Godenail	Boy	+	−	+	+	+	+	5	1
Urban	32	Modern WC, wash	25–36	Normal food	Tap water	Kadamtoli	Boy	+	−	−	+	+	−	3	0
Urban	33	Modern WC, wash	25–36	Normal food	Tap water	Kadamtoli	Boy	+	−	+	+	+	−	4	1
Urban	34	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Kadamtoli	Girl	+	−	+	+	+	−	4	1
Urban	35	Modern WC, wash	49–60	Normal food	Tap water	Godenail	Boy	+	−	+	+	+	−	4	1
Urban	36	Modern WC, wash	49–60	Normal food	Tap water	Godenail	Boy	+	−	+	−	+	−	3	0
Urban	37	Modern WC, wash	25–36	Normal food	Tap water	Godenail	Boy	+	+	+	−	−	−	3	0
Urban	38	Modern WC, wash	49–60	Normal food	Tap water	Godenail	Boy	+	−	+	−	+	−	3	0

Urban	39	Modern WC, wash	49–60	Normal food	Tap water	Godenail	Boy	+	+	+	−	+	−	4	1
Urban	40	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Godenail	Girl	+	−	+	−	+	−	3	0
Urban	41	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Godenail	Boy	+	−	+	−	−	−	2	0
Urban	42	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Godenail	Boy	+	+	−	−	+	−	3	0
Urban	43	Modern WC, wash	37–48	Normal food	Tap water	Godenail	Girl	+	−	+	−	+	−	3	0
Urban	44	Modern WC, wash	49–60	Normal food	Tap water	Godenail	Boy	+	+	+	−	+	−	4	1
Urban	45	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Godenail	Girl	+	−	+	−	+	−	3	0
Urban	46	Modern WC, wash	25–36	Normal food	Tap water	Godenail	Boy	+	−	+	+	+	−	4	1
Urban	47	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Godenail	Boy	+	−	+	+	+	+	5	1
Urban	48	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Godenail	Girl	−	−	+	+	+	+	4	1
Urban	49	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Godenail	Boy	−	−	+	+	−	+	3	0
Urban	50	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Fatulla	Girl	−	−	+	+	+	+	4	1
Urban	51	Modern WC, wash	49–60	Normal food	Tap water	Fatulla	Girl	+	−	+	+	+	+	5	1
Urban	52	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Fatulla	Girl	+	−	+	+	+	+	5	1
Urban	53	Modern WC, wash	49–60	Normal food	Tap water	Fatulla	Girl	+	−	+	+	−	+	4	1
Urban	54	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Fatulla	Girl	+	−	+	+	−	+	4	1
Urban	55	Modern WC, wash	13–24	Breast milk and soft food	Tap water	Fatulla	Boy	+	−	+	+	−	+	4	1
Urban	56	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Fatulla	Boy	+	+	+	−	−	+	4	1
Urban	57	Modern WC, wash	25–36	Normal food	Tap water	Fatulla	Girl	+	−	+	+	−	+	4	1
Urban	58	Modern WC, wash	37–48	Normal food	Tap water	Fatulla	Girl	+	−	+	+	−	+	4	1
Urban	59	Flush/pit latrine, wash	49–60	Normal food	Tube well	Matuail	Girl	+	−	+	−	−	−	2	0
Urban	60	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Fatulla	Girl	+	−	+	+	+	−	4	1
Urban	61	Flush/pit latrine, wash	37–48	Normal food	Tube well	Matuail	Girl	+	−	+	−	+	−	3	0
Urban	62	Flush/pit latrine, wash	49–60	Normal food	Tube well	Matuail	Boy	+	−	+	−	+	−	3	0
Urban	63	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Siddhirganj	Boy	+	−	+	−	+	−	3	0
Urban	64	Modern WC, wash	25–36	Normal food	Tap water	Fatulla	Girl	+	−	+	+	+	−	4	1
Urban	65	Modern WC, wash	25–36	Normal food	Tap water	Fatulla	Girl	+	−	+	−	+	−	3	0
Urban	66	Modern WC, wash	0–12	Breast milk and soft food	Tap water	Siddhirganj	Girl	+	+	+	−	+	+	5	1
Urban	67	Modern WC, wash	25–36	Normal food	Tap water	Fatulla	Girl	+	+	+	−	+	−	4	1
Urban	68	Modern WC, wash	25–36	Normal food	Tap water	Fatulla	Boy	+	−	+	+	+	−	4	1
Urban	69	Modern WC, wash	49–60	Normal food	Tap water	Fatulla	Boy	+	+	+	+	+	−	5	1
Urban	70	Flush/pit latrine, wash	0–12	Breast milk and soft food	Tube well	Matuail	Boy	+	+	+	−	+	−	4	1

+; gene−specific band positive, −; gene−specific band negative. Pit latrine w/o slab, w/o wash indicates children use a pit latrine without a slab and do not wash hands afterwards; flush/pit latrine, wash indicates children use a flush or a pit latrine and wash their hands after visiting latrine and before eating food; modern WC, wash indicates children use modern toilet, wash hands after defecation and before eating food. Under Category, children were categorized by the numbers of genes detected: 0, 1–3 genes detected; 1, 4–6.

4.3. Personal hygiene and salmonellosis in children

As salmonellosis is also spread by food, personal hygiene habits may have an impact on salmonellosis in children. We, therefore, also sought information about each child's hand-washing habits and whether the household used a pit latrine with or without a slab. Of the rural children, 33 (47.1%) used pit latrines without a slab and did not wash their hands after visiting the latrine or before eating food, and 37 (52.9%) used pit latrines with slabs and washed their hands after defaecation and before eating food; among the urban children, 15 (21.4%) used latrines with flush water and washed their hands after visiting the latrine and before taking food, and 55 (78.6%) used modern toilets and washed their hands after defaecation and before eating food. However, hygiene habits did not have a significant impact on the number of *Salmonella* genes detected (Table 6).

Table 6 Multivariable analysis of *Salmonella* gene based on factors associated with area and hygiene.

Variables		Crude OR	95% CI	Adjusted OR	95% CI
Area	Rural	1	(ref)	1	(ref)
	Urban	4.792	(2.35–9.81)	3.286	(0.93–11.62)
Hygiene	Modern W/C, wash	6.133	(2.38–15.87)	1.867	(0.39–9.07)
	Flush/pit latrine, wash	1.971	(0.79–4.96)	1.4	(0.52–3.8)
	Pit latrine w/o slab, w/o wash	1	(ref)	1	(ref)

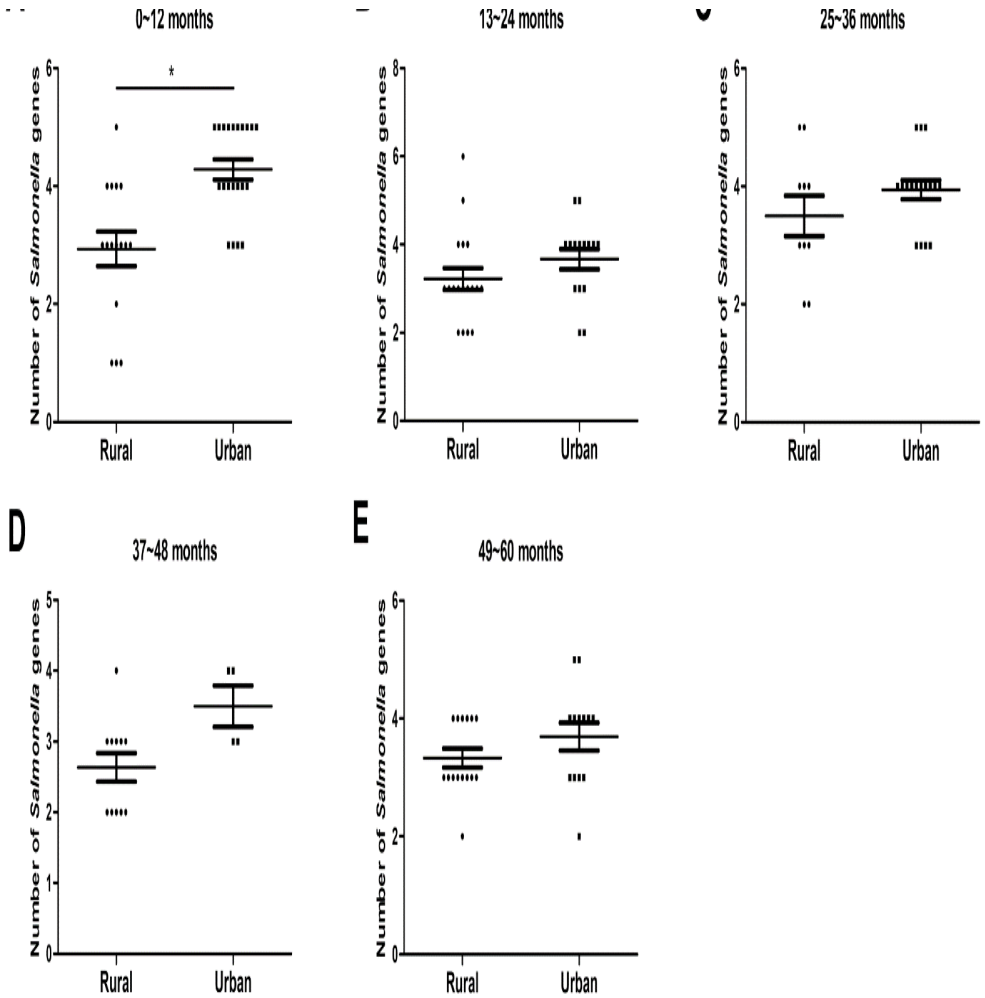
OR: odds ratio, CI: confidence interval. Adjusted OR for *Salmonella* was calculated from the multivariable logistic model, including area and hygiene simultaneously. WC, water closet; w/o, without; wash, handwashing.

4.4. Salmonellosis in children: Does it vary with age and sex?

Ages of the children studied by locality (rural and urban) were as follows: 0–12 months, 16 rural (22.9%), 21 urban (30%); 13–24 months, 18 rural (25.7%), 15 urban (21.4%); 25–36 months, 10 rural (14.3%), 17 urban (24.3%); 37–48 months, 11 rural (15.7%), 4 urban (5.7%); and 49–60 months, 15 rural (21.4%), 13 urban (18.6%). The median ages of rural and urban children were 27 and 24 months, respectively. Among the rural children, there were 38 girls (54.3%) and 32 boys (45.7%), whereas, in the urban group, there were 30 girls (42.9%) and 40 boys (57.1%).

We analysed the data by age group (Figure 5). Of interest, children in the under 12-month urban group had significantly higher Salmonella gene scores than those found in rural samples ($p < 0.001$) (Figure 5A) or other urban age groups. Urban children under one year of age had more *Salmonella* genes (mean, 4.29) than rural children in the same age group (mean, 2.94). By gender subgroup in rural or urban areas, there was no significant difference in numbers of children harbouring *Salmonella* genes (Table 5).

Figure 5 Urban Bangladeshi infants aged 0–12 months have more *Salmonella* genes than older urban children and all children in rural

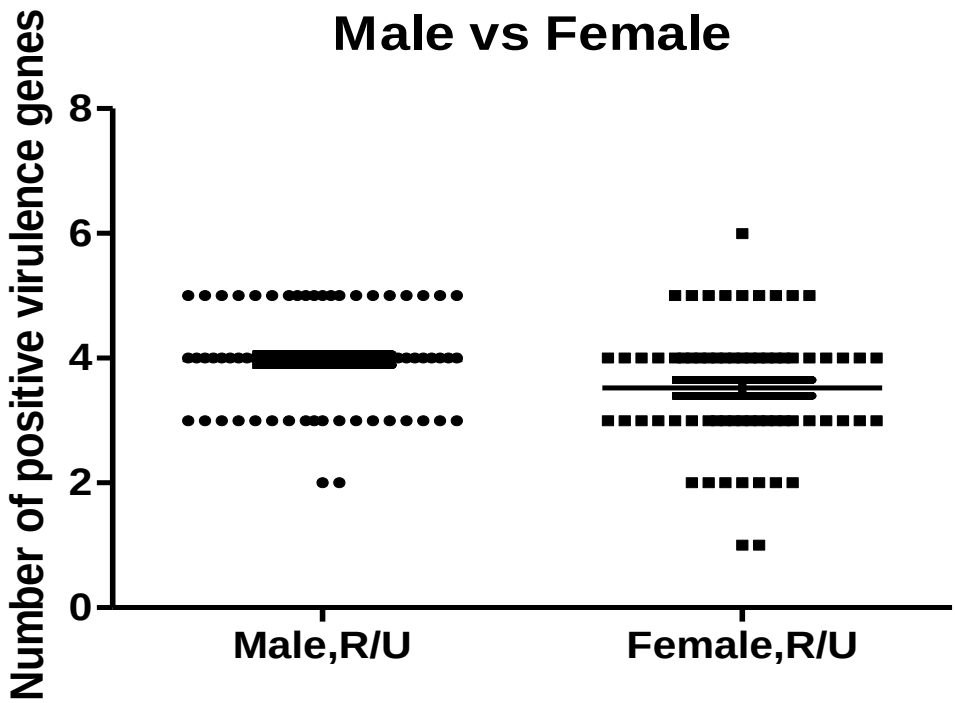


Salmonella genes from samples collected in rural and urban areas were sub-grouped according to child's age and reorganised and analysed by Mann-Whitney Test. (A) 0~12 months; (B) 13~24 months; (C) 25~36 months; (D) 37~48 months; (E)) 49~60 months. Rural (0), Urban (⊿). ***p < 0.001.

4.5. Salmonellosis: Boys and Girls

We studied data to see if gender has a role, i.e., if boys have more incidence of salmonellosis than girls or vice versa. Data suggested that boys suffer more from salmonellosis than girls across the urban and rural areas.

Figure 6. Salmonellosis: Boys and girls

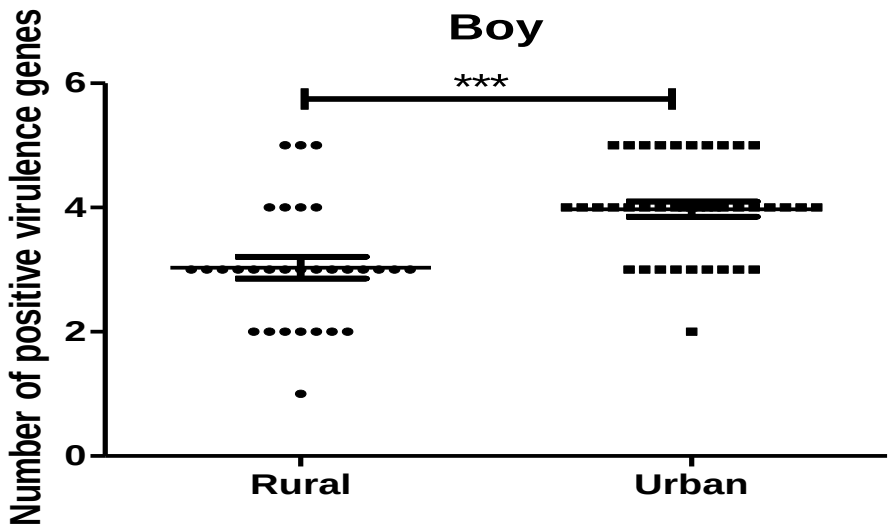


Boy vs Girl: Boys harbour more *Salmonella* virulence genes than girls.
*p<0.05.

4.6. Salmonellosis: In rural and urban boys

We then compared data to see any difference in harbouring virulence genes between rural and urban boys. We found that urban boys harboured more *Salmonella* virulence genes than rural boys.

Figure 7 Salmonellosis: Boys in rural and urban areas

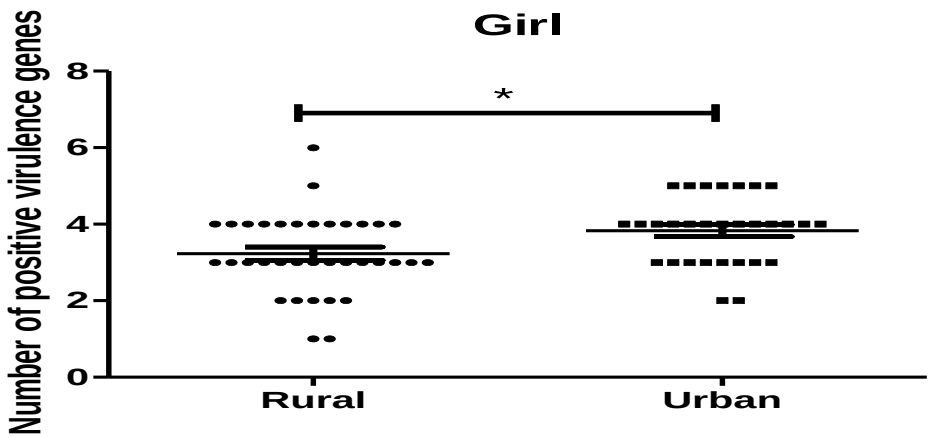


Rural vs urban boy. Urban boys harbour more Salmonella virulence genes than rural boys *p<0.05

4.7. Salmonellosis: In rural and urban girls

We compared data to see if there is any difference in harbouring virulence genes between rural and urban girls. We found that urban girls harboured more *Salmonella* virulence genes than rural girls.

Figure 8 Salmonellosis: Girls in rural and urban areas



Rural vs urban girls. Urban girls harbour more Salmonella virulence genes than rural girls *p<0.05

4.8. Salmonellosis and dietary habit

We investigated the data to find out if children's dietary habit has any impact on their having salmonellosis. We found no significant difference between the two groups of children having breast milk and soft food in one group and having normal food in the other group.

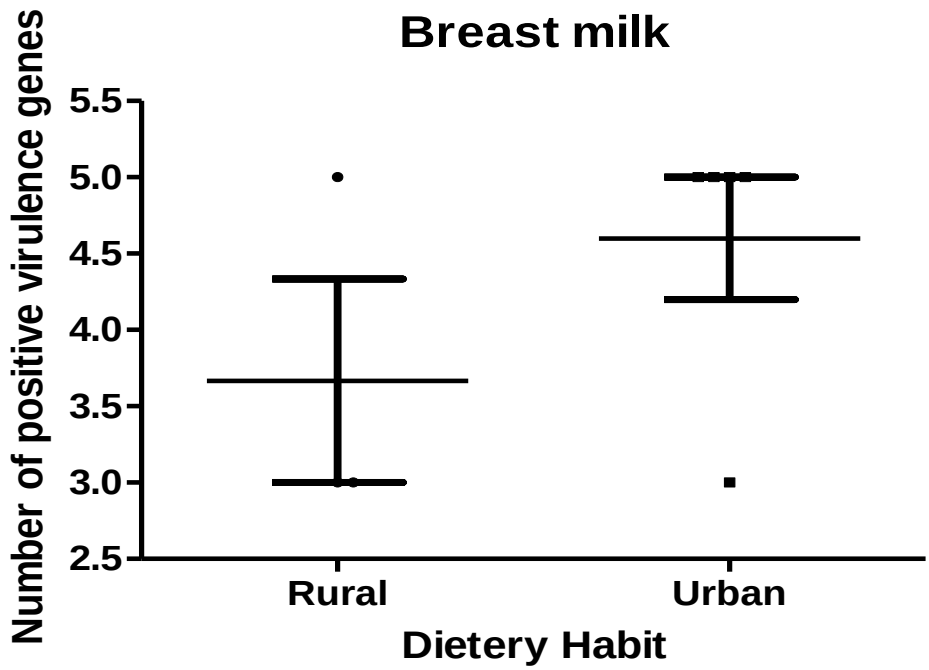
Figure 9 Salmonellosis and dietary habit

Breast milk and soft food vs normal food: No significant difference between the two groups of children having breast milk and soft food in one group and having normal food in the other group.

4.9. Salmonellosis and breast milk in rural and urban children

The study looked at the data to find out if there is any difference in the incidence of salmonellosis in rural and urban children who were fed with breast milk. No significant difference could be noted between the two groups of children.

Figure 10 Salmonellosis and breast feeding



Breast milk in urban and rural children: No significant difference is noted.

4.10. Salmonellosis: Children having breast milk and soft food in rural and urban areas

We further looked into the data to see if there was any difference in the incidence of salmonellosis among rural and urban children who had breast milk and soft food. It was noted that urban children having breast milk and soft food had more virulence genes than rural children having breast milk and soft food.

Figure 11 Salmonellosis: Breast milk and soft food diet in rural and urban children

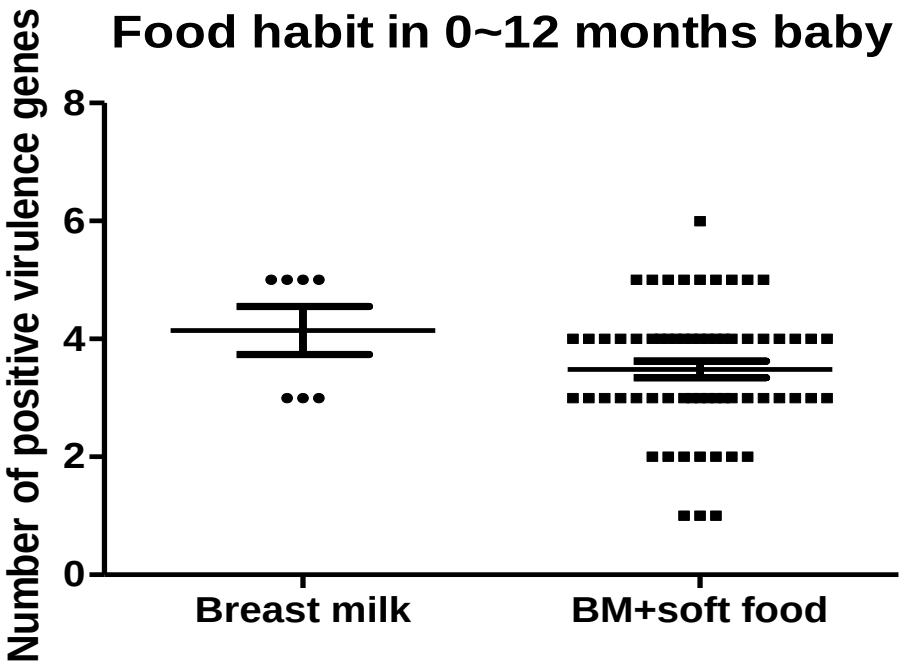
Dietary habits: Significant difference between urban and rural children having breast milk and soft food i. e., urban children harbour more Salmonella virulence genes having same food as rural children *p<0.05

4.11. Salmonellosis: Food habit in the 0~12 months age-group

Since we noted that the children in the 0~12 months age group harboured the highest number of virulence *Salmonella* genes in their stool samples, we investigated if they have any difference in the number of *Salmonella* genes according to their dietary habit. No significant difference in the number of virulence genes was noted in children in this age group having breast milk in one group and breast milk plus soft food in

another group.

Figure 12 Salmonellosis: Food habit in 0~12 months children

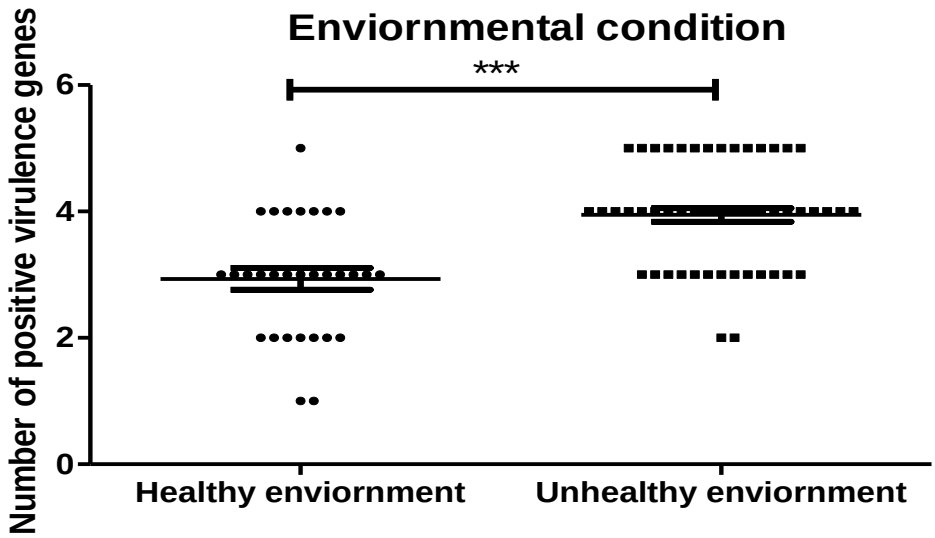


Dietary habit in the age-group of 0—12 months: No significant difference in children in this age-group having breast milk in one group and breast milk plus soft food in another group.

4.12. Salmonellosis: Environmental condition

Several earlier studies suggested that the environment in which children live may impact the incidence of salmonellosis in these children. Our data also found that children living in unhealthy environmental conditions harbor more *Salmonella* virulence genes than children who live in relatively healthy environmental conditions.

Figure 13 Salmonellosis and Environmental Condition



Environmental condition: Children living in unhealthy environmental conditions harbor more Salmonella virulence genes than children who live in relatively healthy environmental conditions.

Discussion

Enteric fever is an acute, systemic infectious disease caused by *S.* Typhi *and S.* Paratyphi A, B, or C, which often manifests with high-grade fever, coated tongue, nausea or vomiting, diarrhea, abdominal pain, and cough [82]. It predominantly affects children and young adults because they either lack natural immunity or experience high levels of exposure to fecal pathogens [83-84]. Each year, approximately 16 million cases of illness and over 153,000 deaths are attributed to enteric fever. However, estimates vary and are uncertain due to the limited number of population-based incidence studies [85].

In addition, enteric fever continues to be an important global health problem, especially in low- and middle-income countries of South Asia, including Bangladesh [83, 85-89]. These low-resource countries experience a high burden of enteric fever because they have limited access to safe drinking water and adequate sanitation and hygiene [83, 90]. Salmonellosis is one of the leading causes of morbidity and

mortality across the world. Typhoid fever occurred in more than 20 million people in the year 2000, and It causes approximately 200,000 death annually [91]. In the Indian subcontinent, Pakistan has the highest incidence (451.17 per 100,000 persons/year) of typhoid fever, followed by India (214.2 per 100,000 persons/year) [92]. In India and Bangladesh, typhoid fever disproportionately affects children, and the highest incidence has been observed among children aged <5 years [93]. In Bangladesh, the incidence of invasive Salmonellosis is high among the residents of the densely populated urban communities in Dhaka and neighboring areas of the Dhaka metropolitan area [94].

A study, reported by Saha et al. between January 2013 and December 2014, found that 1.8% of all blood cultures (279 of 15,917) or 63% of culture-positive cases (279 of 443) were laboratory-confirmed enteric fever cases; 86% (241 of 279) of them were laboratory-confirmed *S.* Typhi, and 14% (38 of 279) were *S.* Paratyphi A [95]. The age of the hospitalized children ranged from 1 day to 18 years with a median of 7 months. A community-based study in an urban

slum in Bangladesh, by Brooks et al., suggested that the overall incidence was 3.9/1,000 persons/year, and the rate was higher in pre-school children aged between 0 and 4 years (18.7 per 1,000 persons/year) [6]. A recent study by Ashraf et al. revealed that the incidence rate is higher in children aged <5 years (10.5/1,000 persons/year) with an overall incidence rate of 2.0/1,000 persons/year [13].

Besides overcrowding and unsanitary urban condition, it was also observed that lifestyle in terms of eating non-home food also has a negative impact on salmonellosis. Rahman et al. found that the prevalence of typhoid fever was high among the school-aged children (66.67%), children habituated with unsafe drinking water (58.33%) and junk foods (72.92%)—some distinct characteristics of urban Bangladesh, for example, of Dhaka city and its adjacent Narayanganj metropolitan areas [96].

It was also observed that unsafe drinking water, a problem in urban cities of Bangladesh, plays a significant role in the higher incidence of typhoid fever in Bangladesh. A study by Rahman et al. found that 62.50% of school-aged children in

Dhaka city who drank piped water without boiling were positive for salmonellosis and 20.83% positive for children who drank piped water after boiling—opposed to just 16.67% positive for children who drank tube–well water—considered safer for drinking in Bangladesh than tap water [97].

Another study by Corner et al. indicated that contaminated surface and piped water could amplify the likelihood of water–borne infections among people living in the Dhaka metropolitan areas, particularly of salmonellosis [98]. They also found that in high–risk areas (overcrowding, poor housing condition, inadequate safe sanitation, etc.), 72.73% of people had low QOL (Quality of Life), 18.19% had medium QOL, and only 9.08% presented high QOL. The study assumed that unplanned urbanization, higher population density, lack of critical urban infrastructures, etc., in Dhaka metropolitan areas had a considerable impact on the transmission and distribution of salmonellosis.

In this study, water samples were not tested for their contamination levels—as that would have involved another extensive research. This instant study rather focused on

examining the stool samples of healthy children from rural and urban areas of Bangladesh. There had been earlier studies on the water contamination levels of Bangladesh. In this study, we used a water test report by Shahidul et al., who found, based on heterotrophic plate count (HPC), that all tap water sources of Dhaka city restaurants were highly contaminated [99]. The HPC of Dhaka WASA (Water and Sewerage Authority) was found to be between 1.2 X 10^4 and 5.4 X 10^4 cfu/ml against the WHO-recommended level of tap water HPC at 100—500 cfu/ml [100]. They found that 90% of the samples were contaminated with one or more of the three potential pathogenic species—*Vibrio*, *Shigella,* and *Salmonella*. Typhoid fever is also highly endemic in Nepal. In recent studies, municipal water in Kathmandu was contaminated with *S.* Typhi and *S.* Paratyphi A. Exposure to these water sources and socioeconomic status were identified as risk factors for *S.* Typhi [101-102]. In a retrospective study in 5 hospitals in Nepal, 1881 cases of typhoid fever were culture-confirmed, with 70% of those cases in children aged <15 years [103].

The study by Rahman et al. also indicated that the availability

of junk food in urban areas, which are not usually found in rural Bangladesh, also contributes to the high incidence of Salmonellosis in urban children [96]. It was found that 79.17% of children who ate both home and junk food were positive for *Salmonella* spp. against 20.83% who were accustomed only to home food.

Thus, this high incidence of salmonellosis was observed in metropolitan areas of Dhaka despite its population' s access to tap water (piped water), civilized sewerage system, and better personal hygiene behavior. While all studies agree that the incidence of virulence *Salmonella* spp. has been higher in urban children due to various environmental factors, those studies so far could not establish any convincing reason for this apparent contradiction, i.e., higher incidence of invasive Salmonellosis in an urban setting with better facilities of drinking water and sewerage system than in rural setting with no or little access to piped water and civilized sewerage system.

In consideration of this literature and view of the higher incidence of salmonellosis in ill-urbanized and overcrowded

metropolitan cities of Bangladesh, the hypothesis of this study, therefore, has been whether urban children harbor more virulence genes of *Salmonella* spp. in their intestines as commensal bacteria that might be responsible for the high incidence of salmonellosis in urban children compared to rural children. The results of this study proved the hypothesis quite convincingly, showing that the intestines of urban children of Narayanganj City Metropolitan area indeed harbor much more virulence genes of *Salmonella* spp. than their counterparts living in rural areas of Sirajganj. The results thus establish the co-relationship between the high incidence of invasive salmonellosis in urban children and the presence of a higher level of virulence genes of *Salmonella* spp. as commensal bacteria in the intestine of urban than in rural children.

The findings also exposed the consequences of the existing unplanned urbanization of Bangladesh characterized by a faulty piped water system and sewerage system that fails to supply safe drinking water to the population living in the metropolitan areas of Bangladesh. As it is reported in the media from time to time, tap water pipes get cross-connected

to the sewerage pipes on a regular basis resulting in contamination of pipe water with sewage—a major source of contamination of tap water in the metropolitan areas of Dhaka and its neighborhood like Narayanganj.

This cross-sectional study was carried out to detect indigenous *Salmonella* spp. in the intestine of healthy rural and urban children of Bangladesh—using a stool as a study sample. Stool contains many different types of bacteria, and as such, conventional PCR of stool usually gives many nonspecific bands. To reduce these nonspecific bands and get clear *Salmonella* spp. bands, nested PCR was used for this experiment for obtaining targeted *Salmonella* bands. Nested PCR is a modification of PCR that was designed to improve sensitivity and specificity. It reduces the non-specific binding of the product due to the amplification of unexpected primer binding sites. Nested PCR involves the use of two primer sets and two successive PCR reactions. The first set of primers are designed to anneal to sequences upstream from the second primers and are used in an initial PCR reaction. Amplicon resulting from the first PCR reaction is used as a

template for the second set of primers and a second amplification step. The sensitivity and specificity of DNA amplification may be significantly enhanced with this technique [104].

Typhoid fever is one of the leading causes of morbidity and mortality among children worldwide. Buckle and colleagues estimated 13.9 to 26.9 million cases of typhoid and paratyphoid worldwide in 2010 [105]. According to the most recent estimates of 2014, approximately 21 million typhoid cases and 222,000 typhoid-related deaths occur annually worldwide [106]. In southeast Asia, the annual typhoid incidence (per 100,000 person-years) varied from 24.2 and 29.3 in sites in Viet Nam and China, respectively, to 180.3 in the site in Indonesia; and to 412.9 and 493.5 in sites in Pakistan and India, respectively.

Altogether, 23% (96/413) of isolates were multidrug-resistant (chloramphenicol, ampicillin, and trimethoprim-sulfamethoxazole) [107].

In Bangladesh, a South Asian country, typhoid fever is endemic. Mortality and morbidity among children suffering

from typhoid fever are also significantly high in Bangladesh. Empirical and phenotypical evidence suggests that *Salmonella* infection incidence is at least twelve times higher in urban areas than in rural Bangladesh (151 incidences in rural areas and 1,900 in urban areas per 100,000). (Reported by Samir Kumar Saha of Dhaka Shishu Hospital, personal communication) [108].

In Bangladesh, the incidence of invasive salmonellosis is high among residents of densely populated urban communities such as Dhaka and neighboring metropolitan areas [109]. A 2005 community-based study in an urban slum in Bangladesh reported an overall incidence of salmonellosis of 3.9/1,000 person-years and a higher rate in children aged 0-4 years (18.7/1000 person-years) [110]. Another study found a higher incidence in children aged <5 years (10.5/1,000 person-years) with an overall incidence rate of 2.0/1,000 person-years [93].

In Bangladesh, typhoid fever disproportionately affects younger children, and the highest incidence has been observed among children aged <5 years [6, 111, 112].

The incidence of invasive salmonellosis is high among the residents of the densely populated urban communities in Dhaka and neighboring areas of the Dhaka metropolitan area [94].

Overcrowding and unsanitary urban conditions, particularly lack of access to safe drinking water, have been attributed to the high incidence of salmonellosis in metropolitan children. These children who drank piped water without boiling in Dhaka city were found positive for salmonellosis [96]. Eating contaminated restaurant food or fast food has also been identified as a major source of Salmonellosis infection in Dhaka city residents. They also found that the prevalence of typhoid fever was high among the children of school-going age (66.67%), children habituated with unsafe drinking water (58.33%), and those eating junk foods (72.92%) [96].

Bangladesh is one of the most densely populated countries globally, with an estimated population of 166 million (Banglade sh Bureau of Statistics, unpublished data). A riverine country —crisscrossed by hundreds of rivers—with monsoon flood and river erosion, a significant number of Bangladesh ' s

population have moved to the cities searching for livelihood. This has created huge infrastructural pressure on the cities. Additionally, Bangladesh's urbanization has been rapid, mostly unplanned, and resource-constrained, which consequently resulted in an inefficient and unsafe sanitary and water-supply situation in the cities. Lack of coordination among different agencies in charge of drinking/household water supply and sewerage/waste disposal has worsened the problem. Several investigative media reports described how Dhaka city's drinking water supply pipes get regularly cross-connected to the sewerage drains—contaminating the supply water in despicable proportions (Nazrul I., unpublished data).

Also, the incidence of nontyphoidal salmonellosis has historically been higher in metropolitan areas of Bangladesh than in rural villages. A study conducted in 1977-1979 found that a total of 214 isolates from blood and stool from metropolitan Dhaka city were positive at 0.32% (out of 66,342 cultures) against 12 positive isolates from rural Matlab area at 0.04% (out of 27,265 cultures) [9].

A recent study by Dr. Samir K Saha of Dhaka Shishu Hospital (children's hospital) found that 64% of all isolates from three Dhaka hospitals were positive for *S.* Typhi as opposed to 42% of isolates from rural hospitals (Saha, K., personal communication). A study by Shirajum Monira et al. on the presence of multidrug-resistant pathogenic bacteria in the gut of healthy young children in Bangladesh concluded that the gut of young children below the age of 5 years was an important reservoir for pathogenic bacteria [10].

Multidrug resistance to first-line agents including ampicillin, trimethoprim-sulfamethoxazole, and chloramphenicol has been followed by reported resistance to fluoroquinolones [89]. Antimicrobial resistance has decreased typhoid fever treatment efficacy and increased treatment cost and risk of complications and death [113-114]. Hospitalization for typhoid fever occurs in 10%-40% of cases. Complications including gastrointestinal bleeding and intestinal perforation occur in 1%-4% of cases [115-116].

Most studies agree on the environmental and infrastructural causes of a high incidence of typhoid fever in young urban

children. However, no study has so far been undertaken to determine whether a pre-presence of *Salmonella* spp. in the intestines of children could be a factor in such differing incidences of typhoid fever between urban and rural children of Bangladesh. If it is found to be true, further research may focus on developing preventive measures in this regard, including through the development of vaccines or through routine screening and early treatment—thus reducing mortality and morbidity of these young children living in developing countries characterized by less investment in public health infrastructures and public healthcare services.

This study was thus designed to examine whether urban children harbor more virulent genes of *Salmonella* spp. in their intestines as commensal bacteria than the children in a rural setting, which might be responsible for a higher incidence of salmonellosis in urban children compared to rural children. This study was carried out through (1) extraction of DNA from the stool of urban and rural healthy children of Bangladesh and (2) molecular detection and characterization of *Salmonella* spp. by nested PCR.

We collected stool samples in both rural and urban settings to compare the composition of gut microbiota of healthy children in two different living settings. We selected Sirajganj, a rural district in north-eastern Bangladesh, a typical Bangladeshi district (zilla), composed of a small town as its headquarters and several sub-districts (upazillas). Some villages have civic facilities such as tube well water and better access to health services; others still rely on traditional water sources (i.e., open ponds). These areas lack municipal sewerage systems and water supplies.

For our urban samples, we selected Narayanganj in the outskirts of the Bangladeshi capital, Dhaka. Some of Narayanganj is on the bank of Shitalakshya River, a tributary of the Brahmaputra River. Samples were collected from children in the Narayanganj City Corporation area, which is relatively well-serviced; however, it is an industrial area and is densely populated. Civic facilities are available, but due to congestion and the mostly unplanned and haphazard development, water lines occasionally are connected accidentally to the sewerage system and supply polluted tap

water to most households. As this is an industrial area, many women work, mostly in factories, precluding their ability to breastfeed their children during their long working hours. Instead, they depend on non-breast milk, such as milk from cows or powdered milk. They maintain relatively good levels of personal hygiene, and most have access to tap water for drinking.

Of the rural children studied, 38 (54.3%) drank water from tube wells, and 32 (45.7%) consumed boiled pond water. Among the urban children, 55 (78.6%) consumed tap water and 15 (21.4%) from tube wells (Table 3). Because Salmonella infection is usually transmitted by water and food, and therefore related to hygiene, we also sought information about each child's hand-washing habits and whether the household used a pit latrine with or without a slab. Of the rural children, 33 (47.1%) used pit latrines without a slab and did not wash their hands after visiting the latrine or before eating food, and 37 (52.9%) used pit latrines with slabs and washed

their hands after defecation and before eating food; among the urban children, 15 (21.4%) used latrines with flush water and washed their hands after visiting the latrine and before taking food, and 55 (78.6%) used modern toilets and washed their hands after defecation and before eating food. However, hygiene habits did not significantly impact the number of Salmonella genes detected (Table 4).

Ages of the children studied by locality (rural and urban) were as follows: 0–12 months, 16 rural (22.9%), 21 urban (30%); 13–24 months, 18 rural (25.7%), 15 urban (21.4%); 25–36 months, 10 rural (14.3%), 17 urban (24.3%); 37–48 months, 11 rural (15.7%), 4 urban (5.7%); and 49–60 months, 15 rural (21.4%), 13 urban (18.6%). The median ages of rural and urban children were 27 and 24 months, respectively. Among the rural children, there were 38 girls (54.3%) and 32 boys (45.7%), whereas, in the urban group, there were 30 girls (42.9%) and 40 boys (57.1%). By gender subgroup in rural or urban areas, there was no significant difference in

numbers of children harboring *Salmonella* genes (Table S1).

We first tested five stool DNA samples from *Salmonella*–culture–positive patients, and all samples produced the expected band sizes (16S rRNA, 215 bp; iroB, 201 bp; hilA, 281 bp; hisJ, 231 bp; invA, 197 bp; Stn, 132 bp) for every *Salmonella* gene assessed by nested PCR (Figure 2A).

We found significantly more amplified *Salmonella* genes in urban samples than in rural samples (Figure 3A and Figure 3B). In this study, the stool samples of urban children contained more *Salmonella* genes (mean, 3.92 per sample) than found in rural children (mean, 3.13 per sample).

Many women in rural Sirajganj breastfeed their children at least until the age of two years. Rural children receive little protein compared to their urban counterparts and primarily eat carbohydrates and vegetables. A study in 2013 found that 80.6% of the respondent mothers in a Bangladeshi village were housewives [117]. The study also found that 70.7% of those mothers breastfed their children, and 75.9% fed colostrum to their babies. Most mothers (92.6%) continued to breastfeed their children even if the child was ill. The study

concluded that more rural women breastfed their children than the corresponding national average.

We first tested five stool DNA samples from *Salmonella*–culture–positive patients, and all samples produced the expected band sizes (16S rRNA, 215 bp; iroB, 201 bp; hilA, 281 bp; hisJ, 231 bp; invA, 197 bp; Stn, 132 bp) for every *Salmonella* gene assessed by nested PCR (Figure 2A). Of the 140 stool samples analysed, seven (3 from rural areas [Sirajganj] and 4 from urban areas [Narayanganj]) were negative for 16s rRNA.

We then used *Salmonella* gene counts (0–6) as determined by positive *Salmonella* gene bands in nested PCR to score all samples (Table S2). We found significantly more amplified *Salmonella* genes in urban samples than in rural samples (Figure 3A and Figure 3B). In this study, the stool samples of urban children contained more *Salmonella* genes (mean, 3.92/participant) than found in rural children (mean, 3.13/participant). As drinking water is an important source of *Salmonella* infection, we compared these differences for each water source. Children who drank tap water had the highest

number of *Salmonella* genes (mean, 3.95/sample), followed by children who drank water from tube wells (urban and rural tube wells, respectively, mean 3.80 and 3.24/sample) and boiled pond water (mean 3.03/sample) (Figure 4). These differences suggest that water sources may affect the prevalence of *Salmonella* genes. However, in the multivariable analysis, the water supply system did not significantly affect the *Salmonella* gene number when other factors were adjusted constantly (Table 3).

We then analyzed the data by age group (Figure 5). Of interest, children in the under 12-month urban group had significantly higher *Salmonella* gene scores than those found in rural samples ($p<0.001$) (Figure 5A) or in other urban age groups. Urban children under one year of age had more *Salmonella* genes (mean, 4.29) than rural children in the same age group (mean, 2.94).

Although our study did not include examining a direct relationship between breastfeeding and less incidence of salmonellosis, we have examined the existing literature on the same and found that breastfeeding indeed positively affects

the incidence of salmonellosis in children. A 1980 study noted vigorous responses of colostral and breast milk cells against *Salmonella* spp. [118]. The study concluded that colostral and breast milk cells were demonstrated to be more active against *Salmonella* than blood neutrophils. Another 2004 study found a strong association between having a liquid diet other than breast milk only and sporadic infant salmonellosis, which suggests that breastfeeding prevents infant salmonellosis [119].

Since Bangladesh is in the tropics, we also considered the possibility that seasonal variation might affect our results. We collected the rural samples in May 2016 and the urban samples in June 2016, thus minimizing the collection time gap between the two sample groups. A study found two seasonal peaks of Typhoidal Salmonella (*S.* Typhi and *S.* Paratyphi A) in urban Dhaka in January and February and from August to November. According to the same study, rural Matlab had a single seasonal peak from August to November, but those seasonal peaks were insignificant [120]. Another study

showed there was no significant seasonal difference between May and June in Asia [121].

This study relied on PCR findings of extracted DNA from stools of healthy children. Isolation of live *Salmonella* from the samples collected would have been desired, but this was not possible as half of our stool samples were collected in remote rural areas, where we lacked laboratory facilities that would have enabled us to isolate live organisms from the samples. This was a limitation of this study.

The study's sample size was also relatively small, which may have been due to the strict sample criterion (clinically healthy children with no history of gastroenteritis and/or antibiotic use in the 6 months before the sample collection) that we followed in this study. Thus, finding the right samples was extraordinarily difficult. We also had budgetary limitations in part caused by working in two locations: Bangladesh for sample collection and DNA extraction and South Korea for PCR.

Finally, we were unable to conclusively establish a direct correlation between the higher presence of *Salmonella*

organisms in the intestines of urban children, who also had a higher incidence of typhoid fever compared with rural children. We anticipate that the study results may be helpful in future studies in areas with a high prevalence of ST/PTF (*S.* Typhi /Paratyphi A). Fast diagnosis of ST/PTF through a non-culture-based method (PCR) would aid the development of preventative measures, including routine screening that can be followed by early treatment - as opposed to the time-consuming diagnosis of ST/PTF through traditional culture methods. This, in turn, should have a positive impact on reducing typhoid fever incidence in both urban and rural young children in Bangladesh and countries with a similar milieu.

The culture of organisms (TCM, the traditional culture method) remains the reference standard for identifying bacteria. Although culture remains the preferred diagnostic method for detecting bacteria, it is time-consuming. It may fail to isolate a particular organism due to external factors, such as the absence of high specificity or sensitivity. This study also states that only 40-60% of blood cultures from

patients in the early phase of infection are positive [122]. Molecular detection methods, on the other hand, are suitable for rapidly identifying pathogens in human excreta as these methods are highly sensitive. PCR can detect minute quantities of the DNA of specific pathogens through amplification of a defined DNA segment and by discriminating on one reaction between different organisms even if they are closely related [123-124].

We hypothesized that urban children in Bangladesh may harbour more *Salmonella* genes in their intestines than children in rural areas and that this higher level of *Salmonella* might be why urban Bangladeshi children have a higher incidence of typhoid fever than rural children. Our results agree with earlier findings [96,125]: Urban children in Narayanganj have a higher incidence of salmonellosis than rural children in Sirajganj. We also found that the young urban children of Narayanganj (aged 0-12 months) harbour more Salmonella genes ($p < 0.05$) than rural Sirajganj children in the same age group. Although urban children aged 13-60 months also harbour more *Salmonella* genes than rural

children in the same age group, the difference was significant only for the youngest age group. This finding agrees with earlier studies that found typhoid fever disproportionately affects younger children, particularly children < 5 years old [125–126].

When we looked at children < 5 years old in urban Narayanganj and rural Sirajganj, the difference was not sufficiently strong to reach a definitive conclusion regarding a correlation of the greater number of *Salmonella* genes in the intestines of healthy urban children as a predisposing factor for the higher incidence of typhoid fever. Even though we could not conclusively establish a correlation between greater numbers of *Salmonella* in the intestines of healthy urban children and the higher incidence of typhoid fever in urban than rural children, our findings may be a reference point for future studies and enable a definitive affirmative or negative conclusion to this hypothesis.

Of interest, our study found that the urban children in Narayanganj who drank tap water harboured more *Salmonella* genes than the urban children who drank tube well water and

their rural counterparts in Sirajganj who drank water from tube wells and ponds (boiled water). Of note, the rural people of Bangladesh now usually drink boiled pond water due to years of awareness campaigns. Of the children studied, 38 of the rural Sirajganj children drank tube well water and 32 boiled pond water. Among the urban children, 55 consumed tap water and 15 tube of well water.

In this study, children who drank tap water had the highest number of *Salmonella* genes (average, 3.95 per sample). Urban children who drank tube well water had a mean of 3.80. Rural children who drank tube well water and boiled pond water had means of 3.24 and 3.03 *Salmonella* genes, respectively. These findings are similar to earlier findings [113-115] and reinforce the notion that the lack of safe drinking water remains a major factor in the transmission and distribution of salmonellosis in urban and rural populations, particularly among young children.

Historically, the incidence of non-typhoidal salmonellosis has been higher in metropolitan areas of Bangladesh than in rural areas. A study conducted in 1977-1979 found that of 214

isolates from blood and stool from metropolitan Dhaka, 0.3% of 66,341 cultures were positive compared with 12 (0.04%) of 27,265 positive cultures of isolates from rural areas [10]. Another study reviewed 19,265 blood cultures from an urban pediatric hospital in Dhaka. Of these, 855 (4.4%) were positive by culture for ST/PTF. The same study found 25 (0.2%) of 15,455 blood cultures from a rural hospital in Mirzapur were positive for *Salmonella* [124]. A study of multidrug-resistant pathogenic bacteria in the gut of healthy young children in Bangladesh concluded that the gut of young children below the age of 5 years was an important reservoir for pathogenic bacteria [125].

A previous study suggested that both contaminated surface water and piped water could amplify the likelihood of waterborne infections among people living in the Dhaka metropolitan area, particularly salmonellosis [113]. The authors also found that in high-risk areas (overcrowding, poor housing conditions, inadequate sanitation), 72.7% of residents had low quality-of-life (QOL), 18.2% had medium QOL, and only 9.08% had high QOL. The study found that

unplanned urbanization, higher population density, and lack of critical urban infrastructure in the Dhaka metropolitan area had a considerable impact on the transmission and distribution of salmonellosis.

Another study, based on HPC (Heterotrophic Plate Count), found that all tap water sources of Dhaka city restaurants were highly contaminated [115]. The HPC of the Dhaka Water and Seweage Authority was between 1.2 and 5.4 × 10^4 cfu/mL compared with WHO recommended levels of 100–500 cfu/mL [114]. Some 90% of the samples were contaminated with one or more of the three potential pathogenic species—*Vibrio*, *Shigella,* and Salmonella.

Overcrowding and unsanitary urban conditions, particularly lack of access to safe drinking water, have been linked to the high incidence of salmonellosis in metropolitan children. One study found that 62.5% of school-age children in Dhaka city who consumed piped water without boiling were positive for *Salmonella* as were 20.8% of those who drank piped water after boiling and 16.7% who drank tube well water [118].

Consumption of contaminated water, vegetables, and fast food from restaurants also have been identified as major sources of *Salmonella* infection in Dhaka city residents [116]. This study reported that the availability of junk food in urban areas, which is not usually found in rural Bangladesh, also contributes to the high incidence of salmonellosis in urban children. The study found that 79.2% of children who ate both home-cooked and junk food were positive for *Salmonella* against 20.8% accustomed only to food prepared at home. Another study found that the prevalence of typhoid fever was higher among children of school-age groups (66.7%), children who drank unsafe drinking water (58.3%), and those who ate junk food (72.9%) [118].

Conclusion

As a consequence of environmental, infrastructural, and other reasons, the healthy urban children of Narayanganj in the < 5 years age group harboured more Salmonella genes in their intestines than the rural children of Sirajganj of identical ages. Further research in this area should focus on why the relationship between the presence of *Salmonella* and the incidence of typhoid fever differs in urban and rural healthy children. Moreover, there is a need for studies to concentrate on developing preventive measures such as routine screening and early treatment of salmonellosis to reduce mortality and morbidity of young children. Such measures would be particularly effective in developing countries that lack significant investment in public health infrastructure.

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Abstract (Korean)

배경 방글라데시에서 어린이의 장티푸스 발병률이 시골 지역보다 도시 지역에서 더 높게 나온다. 본 연구자는 건강한 도시 지역 어린이가 건강한 시골 어린이들 보다 살모넬라 관련 유전자 수치가 더 높은지 조사하였다.

실험방법 실험을 위해 대도시, 농촌 지역의 어린이 대변 샘플 각 70개를 확보하여 대변 180~200 mg 에서 DNA 를 추출하였다. 건강한 아이의 대번에서 추출한 DNA 에서 중첩 PCR 통해 살모넬라 관련 유전자를 검사하였다. 16S rRNA 와 살모넬라의 유전자인 Salmonella pathogenicity island I gene (*hilA*), Salmonella enterotoxin gene (*stn*), *invA* gene, Fur−regulated gene (*iroB*), 그리고 histidine transport operon (*hisJ*) 를 표적으로 증폭시켜 비교하였다.

결과 도시 어린이의 대변 샘플 (median 4, IQR 3−4) 에서 시골 어린이 (median 3, IQR 3−4) 보다 더 많은 살모넬라 관련 유전자가 검출이 되었다. 이는 방글라데시 도시 지역의 어린이의 장에 시골 지역 아이 보다 살모넬라 유전자가 더 많은 것을 제시한다. 특히 1살 미만의 도시 지역 어린이의 살모넬라 유병률이 우세하였다. 1살 미만의

도시 어린이 (median 4, IQR 4–5) 에게서 같은 나이의 시골 어린이 (median 3, IQR 2.5–4) 보다 더 많은 살모넬라 유전자가 발견되었다. 물의 공급원이 수돗물인 도시 어린이에서 (median 4, IQR 3–5) 관 우물 (median 3, IQR 2–4), 끓인 연못물을 (median 3, IQR 3–3.5) 섭취하는 시골 어린이 보다 더 많은 살모넬라 유전자가 검출되었다. 그러나 도시 어린이 중 수돗물과 우물이 물의 공급원인 군에서는 살모넬라 유전자의 유의성이 없었다 (median 4, IQR 3–4).

결론 본 연구 결과로 식수 공급 시스템을 포함한 도시 환경으로 인해 시골 어린이 보다 도시 어린이에게 장에서의 잠재적 병원성 살모넬라균의 감염이 높다는 것을 알 수 있었다.

핵심어: 살모넬라 유전자, 방글라데시, 식수 공급 시스템, 도시 어린이, 시골 어린이, 장티푸스

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