DOI: https://doi.org/10.53350/pjmhs2216541

ORIGINAL ARTICLE

Association of Drinking Water and Enteric Fever: A Disguised Source of Infection

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ABSTRACT

Background: Enteric fever is an illness caused by *Salmonella enterica serovars Typhi* and *Paratyphi A* and *B*. The mode of transmission is the ingestion of contaminated food and water.
Aim: To check that whether drinking water is the source of infection or not.
Methodology: 202 water samples were collected from the various areas of Lahore. 120 samples were taken from houses and the neighbourhood of confirmed typhoid patients and 82 samples were randomly collected. The samples were centrifuged, enriched and then subcultured on XLD media. The isolated colonies were identified by biochemical reactions. The sero-typing confirmed *Salmonella* species were done.
Results: The results showed that 23% of the drinking water in Lahore was fit for drinking, 76% samples were contaminated. The organisms isolated were *Serratia* spp. 22%, *Enterobacter* spp. 22%, *E. coli* 16%, *Citrobacter* spp. 12%, *Pseudomonas* spp. 9%, *Proteus* spp. 6%, *Klebsiella* spp. 4%, *Salmonella Paratyphi A* and *Salmonella Rubislaw* as 1%. No *Salmonella Typhi* was isolated from any water sample.
Conclusion: The presences of large coliforms in drinking water were indicative of sewerage contamination. The *Salmonella Paratyphi A* might be source of infection in that specific area but overall the results suggested that drinking water of Lahore was not acting as a source of infection for Enteric caused by *Salmonella Typhi.*
Keywords: Water-borne , Infection, Enteric fever, coliforms.

INTRODUCTION

Salmonella species are a member of family '*Enterobacteriaceae*'. *Salmonella Typhi* is an encapsulated Gram negative rod, non-spore forming and motile. *Salmonella enterica* and *Salmonella bongori* are the two species causing human infections[1]. Within *Salmonella enterica* subspecies, serovars *Typhi* and *Paratyphi A* are the major cause of enteric fever (also known as typhoid fever).

Enteric fever is a significant public health issue in the developing and under developed countries[2]. Worldwide, the incidence of *Salmonella Typhi* cases is about 21 million but the rate of morbidity and mortality is very high in the endemic regions i.e, Southeast Asia and Sub-saharan Africa[3]. The rate reported is around 80% in Asia and 20% in Africa and Latin America[2]. Pakistan also has a high incidence of typhoid fever with significant levels of morbidity and mortality. Bacteremia in children is recorded as high as 1000 positive cases per 100,000 children in Karachi[4].

An important vehicles of transmission is the natural waters[5]. *S. Typhi* can be acquired by drinking / swimming in contaminated water and eating food washed with contaminated water[1]. Majority of outbreaks in the developed countries are due to the intake of untreated or inefficiently treated water. Reports have estimated that approximately 1.1 billion people use unsafe water worldwide. This unsafe water, sanitation and hygiene results in 88% of diarrheal disease in the world. In many areas, the spread of enteric fever is also as a result of ingestion of contaminated food caused by indirect contamination. This indirect contamination can occur while irrigation and even while washing the food with contaminated water[5].

In 2017, an outbreak of XDR *S. Typhi* was reported in Sindh, Pakistan. After the higher incidence of XDR cases, the water supply of the neighborhood of the cases was screened for the presence of *S. Typhi*. Polymerase Chain Reaction of the water sample indicated water contamination which was acting as a reservoir for the outbreak[4].

The purpose of this study was to identify the areas with high incidence of typhoid patients' in Lahore and to check the occurrence of *S. Typhi* in the drinking water of respective areas. This presence of *S. Typhi* will indicate whether water is the source of infection or not.

Received on 11-10-2021
Accepted on 22-04-2022

METHODOLOGY

Sample collection: After IRB permission, forty blood culture proven *S. Typhi* cases were questioned regarding variables such as dietary habits, and pets and detailed information about the source of water intake and whether the water was treated or not before consumption[5]. Then, a visit to the household and neighboring area of the cases was done and water samples were collected from tap[4]. From these 40 blood culture proven cases 120 water samples were taken. 82 water samples were taken randomly from different parts of Lahore. 500mL of water sample was collected in two 250mL sterile glass bottles. The glass bottles were transported to the microbiology laboratory in an icebox by maintaining a temperature of 4°C. The sample was analyzed as soon as it reached the laboratory[5].

Sample processing: The 500mL water sample was divided into ten 50mL falcon tubes. Centrifugation at 3940rpm for 30 minutes was carried out of each falcon tube. The supernatant was removed and the sediment was added in the enrichment media (Selanite F Broth). The bottle was incubated for 8 – 10 hours at 37°C[6]. The sample was sub-cultured on the Xylose Lysine Deoxycholate medium and incubated at 37°C overnight. The isolated colonies were obtained after overnight incubation. All colonies were Gram stained. The colonies consisting of Gram negative rods were tested biochemically.

Catalase test was performed on all of the Gram negative rods. Then oxidase test and tube biochemical reactions (Citrate test, Triple Sugar Iron Test, Urease Test, Motility test, Indole Test, Methyl Red Test and Voges Proskauer Test) were performed. For each biochemical test a known positive and a known negative control was performed. Isolates tested positive for *Salmonella* species on Tube biochemical reactions were again tested via API20E for confirmation.

After the biochemical confirmation of the *Salmonella* species, Serotyping was performed for the serovars confirmation of the *Salmonella* species.

RESULTS

Total 202 water samples were tested. Growth upon subculture were observed in 153 water samples, implying a percentage of 76% indicating contamination and that drinking water was not fit for drinking. The water sample from the *Farooq Ganjh* area was tested

positive for *Salmonella* species, upon serotyping the isolates were confirmed as *Salmonella Paratyphi A* and *Salmonella Rubislaw.* No *Salmonella Typhi* was isolated from any water sample. A number of organisms were isolated from the water samples (Table 1) (Figure1).

Serratia species and *Enterobacter* Species were the most isolated organisms, then being the *Escherichia Coli, Citrobacter* species, *Pseudomonas* Species, *Proteus* Species, *Acinetobacter* species, *Klebsiella* Species and *Providencia* Species.

Table 1: The table illustrates the percentage of water samples which were positive for microbial contamination.

Name of Bacteria	Frequency	%age
Escherichia coli	24	15.59
Salmonella Rubislaw	1	0.54
Salmonella Paratyphi A	1	0.54
Klebsiella spp.	6	3.76
Enterobacter spp.	33	21.51
Proteus spp.	10	6.45
Acinetobacter spp.	8	5.38
Providencia spp.	1	0.54
Serratia spp.	34	22.04
Citrobacter spp.	18	11.83
Pseudomonas spp.	13	8.60
Bacillus spp.	5	3.23
Total	153	100

22% (41 isolates) and then the *Enterobacter* spp. 22% (40 isolates). The third most frequently isolated spp. was *E. coli* 16% (29 isolates). Others were *Citrobacter* spp. 12% (22 isolates), *Pseudomonas* spp. 9% (16 isolates), *Proteus* spp. 6% (12 isolates), *Acinetobacter* spp. 5% (10 isolates), *Klebsiella* spp. 4% (7 isolates), *Bacillus* spp. 3% (6 isolates) and *Providencia* spp., *Salmonella Paratyphi A* and *Salmonella Rubislaw* as 1%.

Figure 1: Pie chart representing the percentages of the various isolated organisms. This pie charts represent that the isolated *Serratia* spp

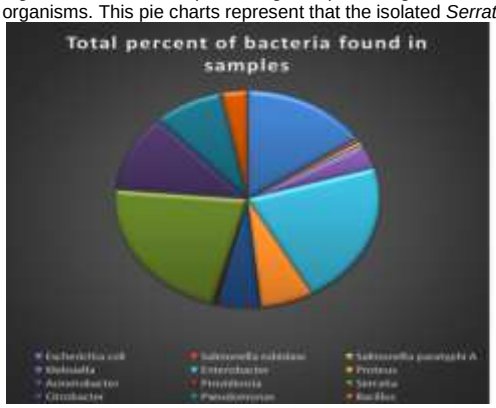

Figure 2: Geographical distribution of samples taken from the different areas of Lahore.

Indicates the location where the sample was taken from.

DISCUSSION

The water samples were collected from September 2020 to January 2021 from various areas of Lahore (Figure 2). The locality was surveyed and the hotspot areas were also targeted. The aim was to look for the presence *S. Typhi* in the drinking water supply of Lahore. Hence, enrichment media was included in the methodology before subculture upon a selective solid agar medium. This technique is a Gold Standard for the extraction of *Salmonella* species from fecal specimen. As the contamination of drinking water is majorly because of mixing of municipal sewerage / waste with the drinking water pipelines[7].

The present study observed the presence of one *S. Paratyphi A* and one *S. Rubislaw* in the drinking water of Farooq Ganjh area, located in Lahore. The presence of *S. Paratyphi A* confirmed that the contaminated water was the main source of infection for the people residing in that area. The results were in accordance with study carried out in Tunisia, which stated that primarily the serovars *Paratyphi* and serovars other than *Typhi* are isolated from the environmental sources[7]. There are many studies conducted in the past which have isolated *Salmonella* species from drinking water. Hsu et al reported 10 *Salmonella* isolates from 116 drinking water samples[1]. Potgieter observed the presence of *Salmonella* species from all of the household water samples from Limpopo province in all seasons[8]. In Nigeria, 4.35% were identified as *S. Typhi* from the drinking water of Ogbomoso North and 15.79% from Ogbomoso South by the similar technique[9].

As discussed earlier, one *Salmonella Rubislaw* was also isolated from our samples. *S.Rubislaw* is a non-typhoidal *Salmonella* species and is the most common *Salmonella* serovars present in the water. *S. Rubislaw* is reported as the 11th most common *Salmonella* spp. causing disease[10]. This non-typhoidal *Salmonella* species is responsible for bacteremia in immunocompromised individuals and children. Other disease include self – limiting diarrhea[11]. In Canada, a study concluded that 29 water samples out of 342 water samples were positive for *Salmonella* species. Within these positive samples *S. Rubislaw* was isolated from 21 water samples[12]. In Ghana, 398 water samples were tested for the presence of coliforms. This study revealed that out of 392 water samples, 26 water samples were positive for *Salmonella* contamination. Within these 4 were *S. Rubislaw*[11]. The presence of *S. Rubislaw* in all of the above studies indicated that ingestion of this contaminated water will be a health hazard for the humans[12].

Santo et al, studied the survival rate of four different species of *Salmonella* in drinking water. He concluded that the species remain viable even after 45 days and all the species respond to the addition of nutrients and growth[13]. Liu stated that in presence of adequate pH and temperature *Salmonella* spp. can survive in environmental sources for 30 days. As for *S. Typhi*, it can easily survive in water till 3 weeks and can easily be resuscitated upon addition of nutrients[14]. These studies conclude that *Salmonella* spp. can easily survive in drinking water for lengthy time span and upon enrichment these can easily be isolated.

Increasing number of patients of XDR *S. Typhi* were observed in Lahore in 2018 - 2020. Testing of the drinking water was done to check if it's the source of the disease. On testing no *S. Typhi* was isolated but two pathogenic *Salmonella* serovars were isolated. The incubation period of *S. Typhi* is 10 – 14 days and the patient may develop symptoms after 6 – 30 days of bacterial ingestion[3]. As there is a big time gap between the outbreak and the time at which water samples were taken, *S. Typhi* might not have survived in the water. Thus, it was concluded that the drinking water of Lahore might act as a single time source but is not acting as a constant source of typhoid caused by *S. Typhi* infection. Probably the main source of spread is mainly foodborne. Infected humans / carriers can give shelter to the bacteria for a long duration of time ranging from months to years.

These carriers / asymptomatic individuals shed the bacteria in the environment, contaminating food/water acting as a source of transmission of disease[7]. The carriers / food handlers working at the restaurants or food outlets may be the main source for the spread of the disease. For this purpose, further investigations should be done on this aspect.

Along with the isolation of the above mentioned *Salmonella* species, many of different species of bacteria were also isolated. *Serratia* species and *Enterobacter* Species were the most isolated organisms. *Escherichia Coli, Citrobacter* species, *Pseudomonas* Species, *Proteus* Species, *Acinetobacter* species, *Klebsiella* Species and *Providencia* Species were also isolated.

Serratia Species are commonly present in water and soil. *Serratia* has a medical importance as an opportunistic pathogen. It is associated with nosocomial infections, such as transfusion associated bacteremia and urinary tract infections. Endocarditis in drug addicts has also been reported [15]. In Germany, it was reported that out of 31 water samples 21% were positive for *Serratia* spp.[16]. In the present study 22% of water samples were positive for *Serratia* spp.

Enterobacter is also a common resident of water, soil and in the environment. *Enterobacter* is mostly causes urinary tract infections and nosocomial infections in the immunosuppressed individuals. In the recent years, bacteremia caused by *Enterobacter* has also been reported. Out of 4.7% bloodstream infections, 3.1% were caused by *Enterobacter*[7]. A very high percent of *Enterobacter* were also isolated form the water supplies of Mozambique. 36.36% of *Enterobacter cloacae* and 27.27% of *Enterobacter asburiae* were isolated[17]. 21.5% of water samples were positive for *Enterobacter* spp. in the present study indicating a health hazard.

Escherichia Coli is the most integral niche in the human intestinal flora. This is also termed as an indicator of fecal contamination in water. The *E. coli* is responsible for various intestinal and extra-intestinal infections in humans[7]. Different strains of *E. coli* are responsible for different features upon ingestion. The most common disease of *E. coli* is traveler's diarrhea. It is produced by the *Enterotoxigenic E. coli.* In South Africa the presence of *Enteropathogenic E. coli* and *Enteroaggregative E. coli* in their drinking water supply were observed. These can result in the outbreak of diarrheal disease or critical illness in the immunocompromised individuals[8]. In the present study 15.6% of the water samples were positive for *E. coli*. The strain of *E. coli* was not identified as it was not the objective of our study but the presence of *E.coli* in our drinking water samples indicated fecal contamination of water.

Citrobacter, a Gram negative rod and a resident of human intestines, is also wide spread in environment especially water and soil. This dissemination in environment is mainly through sewerage water. It is also an intestinal pathogen due to its ability to produce enterotoxin[7]. *Citrobacter* has also been isolated from multiple brain abscesses but in neonates it is considered as an infrequent cause of bacterial meningitis[17]. 11.9% of *Citrobacter* spp. were isolated from the drinking water samples in the present study. Although *Citrobacter* is regarded as an opportunistic pathogen but its presence in the drinking water is worrisome.

Klebsiella is also a member of intestinal flora but it is also a pathogen for humans. It causes nosocomial infection. Nosocomial infections are spread through the hands of healthcare workers. *Klebsiella* is also responsible for pneumonia, urinary tract infections and liver abscesses. It also causes bacteremia and neonatal sepsis[7]. *Klebsiella* has been reported in the drinking water by a number of studies. A study conducted in Kathmandu, Nepal reported 18 samples positive for *Klebsiella* out of 100 ground water samples[18]. Only 3.76% of the water samples in our study were positive *Klebsiell*a spp. posing a threat towards the wellbeing of the population.

The importance of the organisms such as *Citrobacter, Enterobacter, Klebsiella* and *Serratia* species in drinking water is that they also act as a source of antibiotic resistance spread[19].

Proteus, a Gram negative rod, a member of *Enterobacteriaceae* is an inhabitant of soil and water. The existence of *Proteus* spp. in water is an important indicator of the fecal contamination. The main concern of *Proteus* presence in water is its highly antibiotic resistant strains. Although, *Proteus* is an opportunistic pathogen, it is responsible diarrhea, urinary tract infections and even wound infections[20]. In the present study 6.45% of the water samples were positive for *Proteus* spp.

A study carried out in Nigeria, analyzed water samples from taps, storage tanks and distributional pipelines. Only one isolate of *Providencia* spp. was isolated from the total water samples[21]. Similarly, in our study only one sample was positive for *Providencia* spp.

Pseudomonas is a Gram negative rod, but doesn't belong from the family *Enterobacteriaceae.* These species are commonly found in the water sample as a contaminant. It has the ability to form biofilms within water pipelines. This causes the persistence of *Pseudomonas species* in water sample[22]. *Pseudomonas* spp. have been reported to impart taste and odor to the drinking water which might make it difficult for ingestion. In the present study, 8.6% of water samples were positive for *Pseudomonas* spp.

Non-coliforms such as *Acinetobacte*r, was also isolated from the various areas of Lahore. *Acinetobacter* is mostly found in tap water and is also responsible for hospital acquired infections. The inhabitance of this organism in the water supplies is mostly linked with these infections. This was confirmed by a study carried in Tokai University Hospital[23]. In the present study, 5.38% of the water samples were positive for *Acinetobacter.*

Bacillus species were the only Gram positive rods which were isolated. *Bacillus* spp. is the most common bacteria present in the environment. It occupies various environmental surfaces, from landscapes to soil and from soil to water[24]. *Bacillus subtilis* is considered to act as a probiotic in humans. It is even thought that it should be added in water and beverages to increase the human health and decrease the age related illnesses[25]. 3.23% of water samples were positive for *Bacillus* spp. in the present study.

CONCLUSION

It is concluded from the present study that drinking water of Lahore contained coliforms and non-coliforms which were widely distributed in the water supply of Lahore. This depicted faecal contamination of the drinking water. The presence of these pathogenic bacteria depicted that these might act as a source of infection for the whole population. Although, *S. Typhi* was not isolated but one sample was positive for *S. Paratyphi A* which represented that this serovars was responsible for Enteric fever in that area till samples were taken.

Acknowledgements: This work was supported by Department of Microbiology, University of Health Sciences, Lahore, Pakistan.

Conflict of interest: There is no conflict of interest among the authors.

Authorship contribution:

Fatima Tuz Zahra: Sample collection, methodology, software, data curation, writing – original draft, formal analysis, investigation, writing - review & editing and project administration.

Sidrah Saleem: Conceptualization, supervision, resources, funding acquisition.

Muhammad Imran: Supervision, writing review, validation.

Nida Javed: Sample collection, methodology, writing review.

Ayesha Ghazal: Methodology, writing review, investigation.

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