Research Article

Isolation & identification of *Shigella* species from food and water samples of Quetta, Pakistan

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Abstract
Shigella species are frequently associated with food and water borne infections leading to acute invasive enteric infections. Annually there are 165 million cases of shigellosis, of which 163 million are in developing countries and the incidence is highest among children. The study was aimed to isolate and identify *Shigella* species from food and water samples. The isolates were identified by using conventional biochemical tests. Total of 100 samples (50 ready-to-eat salad + 50 household water) were randomly collected aseptically. Out of 100 samples analyzed, 27 (27%) were found positive for *Shigella* species. Out of these 27 positive samples, 16 (32%) were from ready-to-eat salad samples and 11 (22%) were from water. Incidence of *Shigella* species in Quetta city water (28%) is higher as compared to water collected from outside the city which is (16%). *Shigella flexneri* was the most frequent isolate (70%) observed in this study. The high level of *Shigella* species prevalence was observed during the month of April-June. This study revealed that the use of raw animal manure as fertilizer, irrigation of vegetables with fecal contaminated water, a poor sanitary system and improper treatment of water supplies can increase potential risks to the consumer. Adaptation and application of Hazard Analysis and Critical Control Point (HACCP) can decrease the possibility of contamination and eliminate pathogenic microorganisms. Awareness regarding communicable diseases also helps control shigellosis and other diarrheal disease.
Keywords: Biochemical tests; Quetta; Salad; *Shigella*; Water

Introduction
Shigellosis or Bacillary dysentery, a gastrointestinal disease caused by *Shigella* species, is recognized as a serious health problem throughout the world. It is mostly found in developing countries due to improper

waste management, poor hygienic condition and unsafe drinking water. In industrialized nations, it is mostly due to travel to unindustrialized countries and consumption of contaminated food material [1]. Globally, mortality and morbidity due to shigellosis were found to be highest in children under five years old [2, 3].

Worldwide, *Shigella* is responsible for 80-165 million cases of disease and 600,000 deaths annually, of which 1.5 million in developed countries and 163 million are reported in developing countries [4]. In the United state, about 500,000 cases of shigellosis are reported each year [5, 6]. In Africa, it is estimated that more than 8 million *Shigella* infections occur per year, whereas in Asia 91 million cases and 414,000 deaths occur annually [7].

The first report on the *Shigella* isolation and characterization was published by Kiyoshi Shiga in 1897 [8]. The genus *Shigella* is classified in the family *Enterobacteriaceae*. It is gram negative bacilli, non-spore forming, non-motile, 0.5- 0.7µm in size and facultative anaerobic pathogen that are closely related to *Escherichia coli*. It is differentiated from *Escherichia coli* on the basis of serology, pathogenesis and physiology. *Shigella* species usually ferment sugars without production of gas and lactose, urease and oxidase negative [9].

The *Shigella* genus is divided into four species that are *Shigella dysenteriae* (serogroup A), *Shigella flexneri* (serogroup B), *Shigella boydii* (serogroup C) and *Shigella sonnei* (serogroup D). According to biochemical characterization and serological properties, these species are further distributed into several serotypes, as *Shigella dysenteriae* have 15 serotypes, *Shigella flexneri* have 14 serotypes and subserotypes, *Shigella boydii* have 20 serotypes and *Shigella sonnei* with a single serotype [10, 11]. These species are the etiological agent of Shigellosis also known as bacillary dysentery. The symptoms can range from mild watery diarrhea to severe inflammatory dysentery with the passage of mucoid and bloody stools. The other clinical manifestation includes abdominal cramping, fever, nausea, malaise, vomiting and convulsions. Other complications of shigellosis include septicemia, dehydration, joint pains, hypoglycemia, hemolytic uremia and neurological complications [12, 13].

Mode of transmission is via fecal-oral route and by direct contact with an infected individual. The *Shigella* species are highly infectious, as only 10-100 organisms are enough to cause disease and the bacteria is more resistant to stomach acid and can easily pass through the gastric acid barrier [14].

A combination of antibiotics and oral rehydration can lead to the rapid resolution of disease. Currently, there is no protective vaccine targeting *Shigella*, but several vaccine candidates for *Shigella* are under development including killed, live attenuated, ribosomal and conjugate vaccine [15].

Materials and methods

Sample Collection

A total of 100 samples (50 ready-to-eat salad + 50 household water) were randomly collected from different areas of Quetta, during January 2017 to June 2017. Ready-to-eat salad samples were collected in a sterilized stomacher bag from different shops and water samples were collected in the sterilized Duran bottles from Quetta city and outside the city. After collection, the samples were kept in thermopol box filled with crushed ice and transported to Bacteriology Laboratory, Center for Advanced Studies in Vaccinology and Bacteriology (CASVAB), University of Balochistan for further processing. All samples were processed within 4-5 hour of collection.

Isolation of *Shigella*

Ready-to-eat salad and water samples were processed according to the International Organization for Standardization (ISO) 21567: 2004 [16] with some modification. For isolation of *Shigella* species 25 g of each salad

sample was aseptically removed using sterile scalpel and was transferred into a stomacher bag, and 25ml of each water sample was also transferred into a stomacher bag for processing. The samples were inoculated with 225 ml of *Shigella* Broth (Oxoid, UK) supplemented with novobiocin (0.5 µg/ ml), following homogenization of 1 minute in a stomacher bag and was incubated at 41.5 ºC for 18- 24 hour in an anaerobic environment. After incubation, each sample was streaked onto selective and differential agar plates (Hektoen Enteric Agar (HEA) (oxoid, UK), Xylose Lactose Deoxycholate (XLD) Agar (Oxoid, UK) and MacConkey Agar (MAC) (Oxoid, UK)) and plates were incubated at 37ºC for 18-24 hour. Suspected colonies of *Shigella* from each selective medium were picked, and were streaked onto nutrient agar plates and incubated at 37º C for 18-24 hour.

Identification of *Shigella*

Typical colonies were selected from Nutrient Agar plates and were subjected to slide preparation, Gram staining, microscopic observation and biochemical tests. Pure colonies were identified by biochemical tests, triple sugar iron agar (TSI), Motility test, Urease test, Indole test, L-lysine decarboxylase, L-ornithine decarboxylase, Sodium acetate test, Christensen's citrate test and sugar fermentation test (dulcitol, glucose, lactose, mannitol, raffinose, salicin, sorbitol, sucrose and xylose) (all from Oxoid, UK).
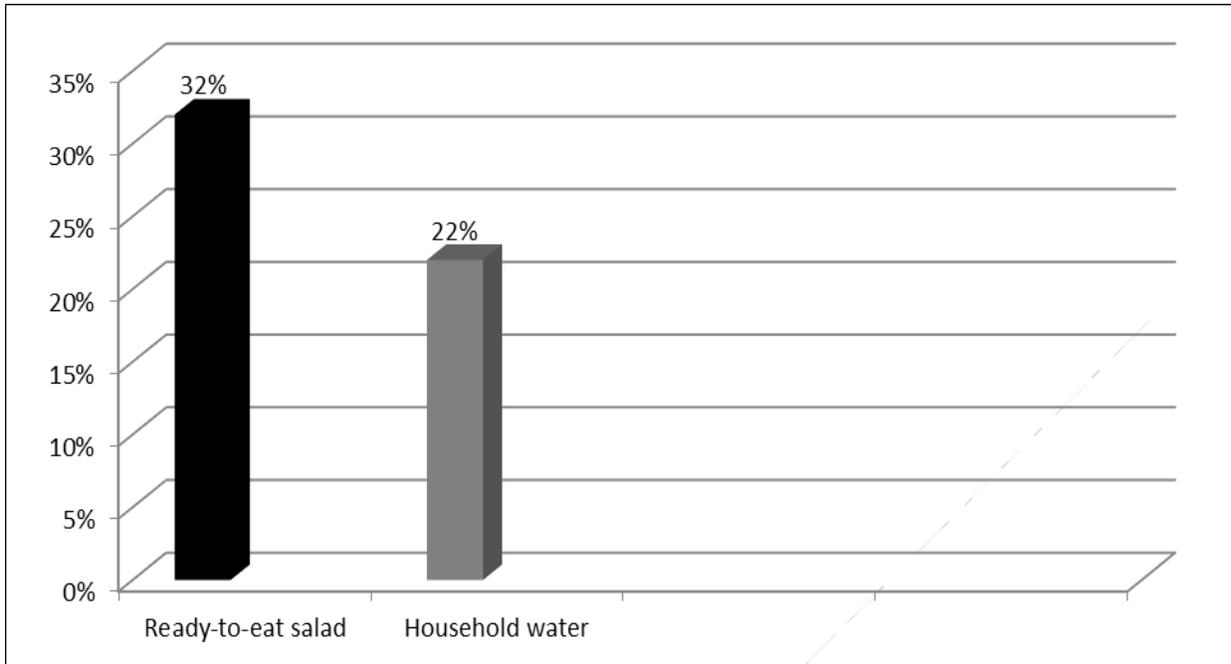
Results

In this study, a total of 100 samples were examined out of which 27 (27%) were found *Shigella* positive. Out of these 27 positive samples, 16 (32%) were from ready-to-eat salad samples and 11 (22%) were from water as shown in (Figure 1). The percentage of *Shigella* species in water samples collected from the Quetta city (28%) was higher as compared to those collected from outside the city (16%) as shown in (Figure 2). The isolated *Shigella* species were found Gram negative bacilli, facultative anaerobic, non-motile and non-sporulating.

The predominant species of *Shigella* identified by biochemical tests, were *Shigella flexneri* and *Shigella dysenteriae*. Among the biochemical tests, negative results were noticed in Urease, Lysine decaboxylase, Ornithine decarboxylase, inability of Sodium acetate and Christensen's citrate utilization. In Triple sugar iron test, isolates produced an alkaline slant and an acid butt with no hydrogen sulphide and gas production for both *Shigella flexneri* and *Shigella dysenteriae*. Both species produced the formation of red ring which indicated the positive reaction (production of indole). Variable results were obtained in tests of mannitol, raffinose and xylose, while fermentation was seen in glucose, dulcitol and sorbitol. Changing of sugar color showed the positive results and there was no fermentation in sucrose, salicin and lactose test. The biochemical tests of *Shigella flexneri* and *Shigella dysenteriae* isolates are shown in the (Table 1). The percentage of *Shigella flexneri* was (26%) and (12%) in ready-to-eat vegetables and water, respectively. In contrast, the percentage of *Shigella dysenteriae* was (6%) and (10%) in ready-to-eat vegetables and water, respectively as shown in (Figure 3).

The month wise prevalence rate of *Shigella* was minimum (12%) in samples collected during January to March, while maximum (39%) in samples collected during April to June as shown in (Figure 4).
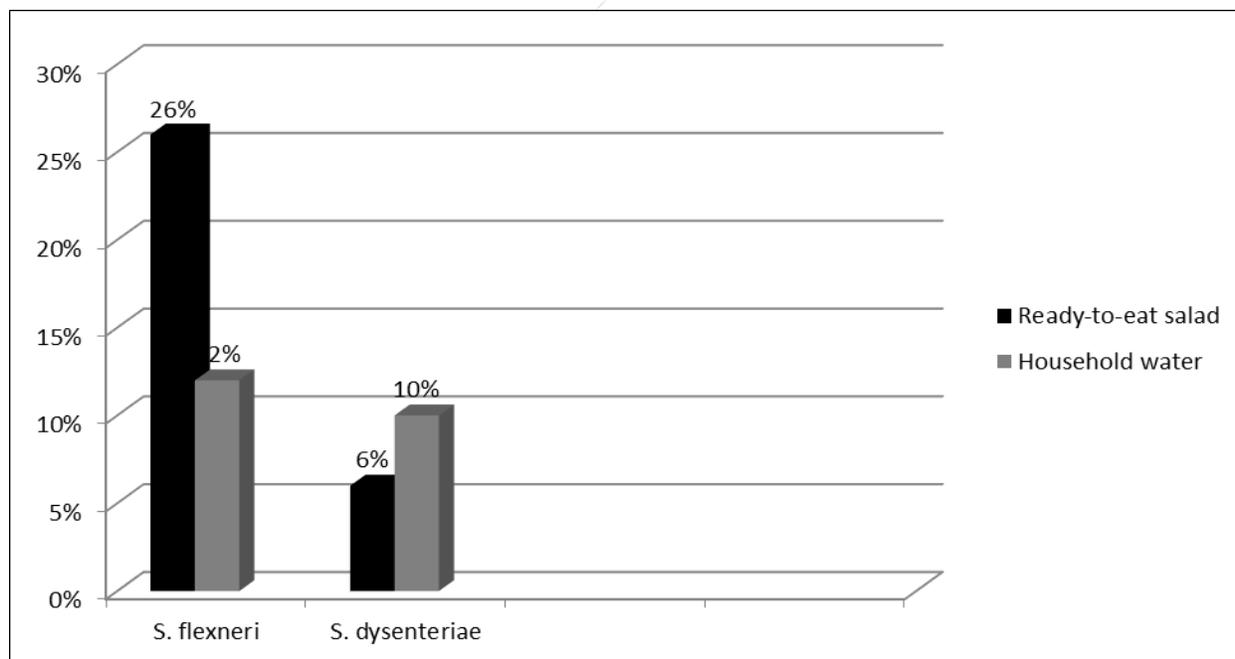
**Figure 1.Prevalence of *Shigella* in ready-to-eat salad and household water**
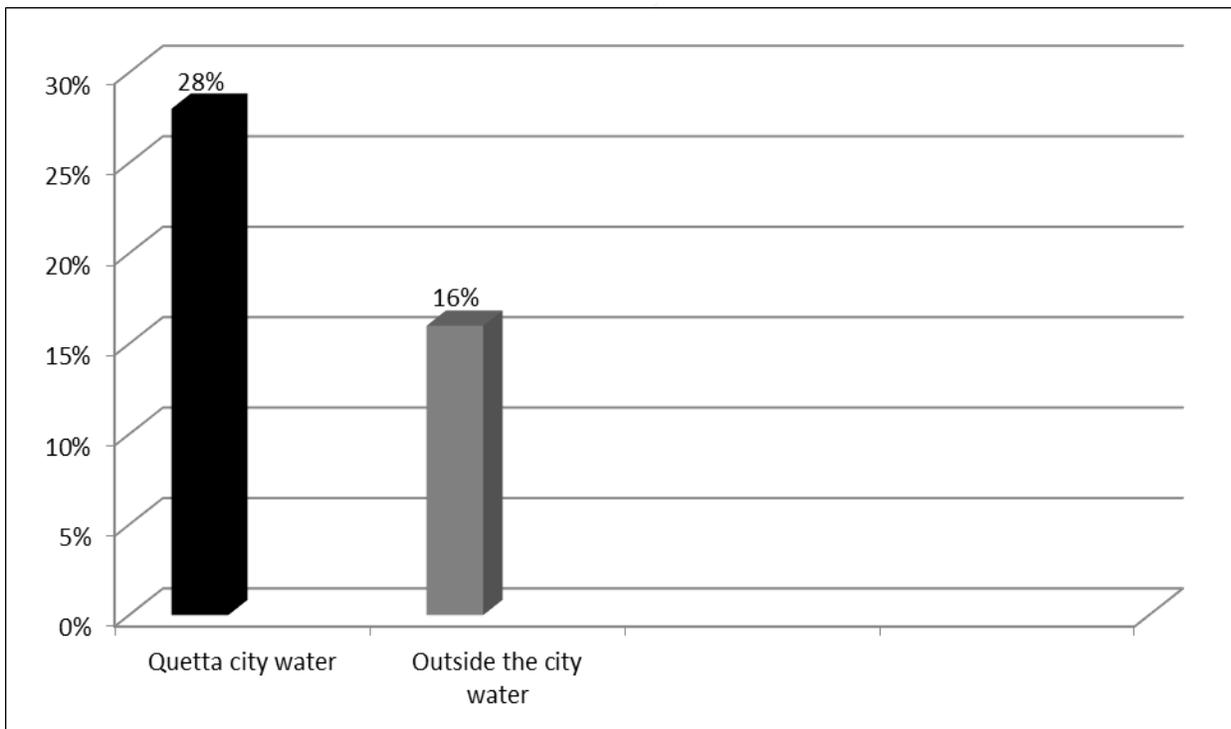
Figure 2. Prevalence of *Shigella* in Quetta city water and outside the city water

Table 1. Result of Biochemical tests for Identification of *S. flexneri* and *S. dysenteriae*.

Biochemical Tests	*Shigella flexneri*	*Shigella dysenteriae*
H$_2$S from TSI	_	_
Gas from glucose (TSI)	_	_
Motility	_	_
Urease	_	_
L-Lysine decarboxylase	_	_
L-Ornithine decarboxylase	_	_
Indole formation	+	+
Sodium acetate	_	_
Christensen's citrate	_	_
Dulcitol	+	+
Glucose	+	+
Lactose	_	_
Mannitol	+	_
Raffinose	+	_
Salicin	_	_
Sorbitol	+	+
Sucrose	_	_
Xylose	_	+

Figure 3. Percentage of *Shigella flexneri* and *Shigella dysenteriae* in the ready-to-eat vegetables and water samples
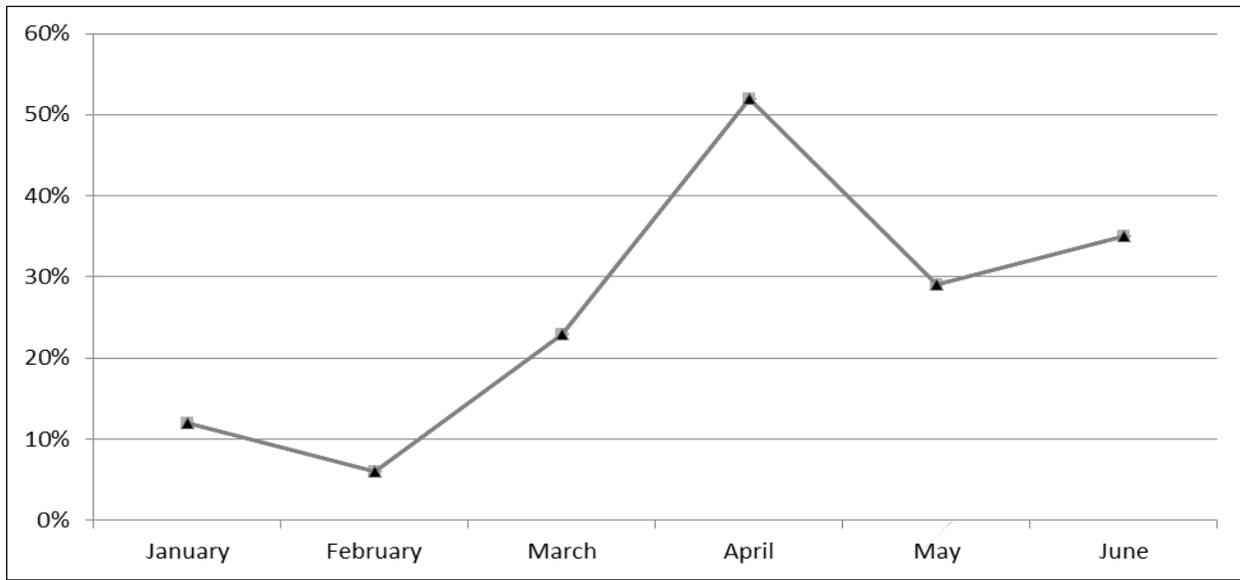
Figure 4. Month wise prevalence rate of *Shigella*

Discussion

Shigellosis is primarily a food and water-borne disease in developing and developed countries. The frequency of *Shigella* species and prevalence of shigellosis varies in different regions of the world. In the present study a total of 100 samples (50 ready-to-eat salad + 50 household water) were analyzed during a period of January to June 2017, out of 100 samples *Shigella* species were isolated and biochemically characterized from 27 samples. Out of these 27 positive samples, 16 (32%) were from ready-to-eat salad samples and 11 (22%) were from water. The results of the present study correlate with the findings of Biniam *et al*. [17] in Ethiopia, who isolated (37%) of *Shigella* species from ready-to-eat salad. Study carried out by Mokhtari *et al.* [18] reported *Shigella* mostly from raw vegetables in Nabeul and Tunisia. There was a high prevalence rate of *Shigella* species within the samples of ready-to-eat salad. This shows the use of unsafe water for washing and sprinkling the vegetable to keep them fresh.

According to the study conducted in India by Joy *et al*. [19], 66.6% of the ready-to-eat salads were contaminated with *Shigella* species, which is higher than the current study. In Nigeria (5.6%) *Shigella* was isolated from ready-to-eat vegetables by Onyemeluk and Njokuobi, [20] which is lower than the present study. These findings were in contrast with the study reported by Soriano *et al*. [21] in Spain, where *Shigella* was not detected in any of the ready-to-eat salad served in Spain University restaurants. Several researchers have concluded that several outbreaks of shigellosis were associated with the consumption of fresh products that are prepared by hand and are served uncooked or raw [22-24]. In addition, previous research data indicated that consumption of unhygienic food is the most common contributing factor in shigellosis in underdeveloped and developed nations [25-27]. The detection of *Shigella* species from ready-to-eat salad revealed the inadequate safety and quality of these products.

In current study the percentage of *Shigella* species isolated from the household water was (16%). The results of present study are consistent with study reported by Rasel *et al*.

[28] in Bangladesh, who isolated *Shigella* species from surface water. A study by Ahmed *et al*. [29] observed 71.0% prevalence of *Shigella* species in Rawalpindi, Islamabad region in Pakistan, from drinking water sample of different dams and related filtration plant, which is higher than the prevalence in household water of current study. A study conducted in Yaounde, Yongsi *et al*. [30] identified 1242 isolates of *Enterobacteriaceae* family from a variety of drinking water, of which *Shigella* species had 0.24% incidence, which is lower than the present study. The finding of this study confirm the prevalence of *Shigella* species in household water and indicates, that it is due to poor sanitation, mixing of sewage water with fresh water and due to the contamination of fresh water with fecal material.

In this study two species of *Shigella* were isolated, *Shigella flexneri* and *Shigella dysenteriae*. *Shigella flexneri* was the most frequent species in the current study. Similar findings were noted in a study carried out in India by Dhodapkar *et al.* [31]. According to previous studies conducted in Egypt, Iran, India [32-34] and a multicenter study by Seliden *et al*. [35] done in Bangladesh, Pakistan, China, Indonesia and Viet Nam *Shigella flexneri* was found as the most frequent species. In contrast, a study conducted in Iran and Thailand where *Shigella sonnei* was the most frequently isolated serotype [35, 36].

The results of our study about month wise distribution showed that the prevalence rate of *Shigella* was higher in April, followed by June and May. Similar findings were reported by Hossain *et al*. [37] who observed high prevalence in April and May in Bangladesh. Various research studies claim that the seasonal tendency of shigellosis is summer [32, 38, 39]. In April, the temperature rises and summer season starts so the cases of shigellosis occurred more than the other months. The high prevalence in summer

indicates that temperature and pH of water are also responsible for the prevalence of *Shigella* during the summer season.

Conclusion
The study concluded that the use of raw animal manure as fertilizer, irrigation of vegetable with fecal contaminated water, a poor sanitary system and improper treatment of water supplies can increase the threat of contamination of ready-to-eat salad and water. To control shigellosis good hygiene, safe handling and processing of food, using clean cutting boards for vegetable, adequate cooking of food, properly washing of raw vegetables before serving, use of boiled water and protection of food from flies are recommended. Awareness regarding communicable diseases also helps control shigellosis and other diarrheal disease.

Authors' contributions
Conceived and designed the experiments: A Samad, F Abbas & Saima, Performed the experiments: Saima, Analyzed the data: M Rizwan, M Naeem, O Pokryshko & S Diaconescu, Contributed materials/ analysis/ tools: M Yousaf, S Saifullah, Y Hassan & M Zahid, Wrote the paper: Saima & Roomeela.

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