Microbiological Quality of Street Vended Drinking Water in Dhaka City and Screening for Antibiotics Resistance of Isolated *Salmonella* spp and *Pseudomonas* spp.

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Received 19 January 2014, accepted in revised form 8 April 2014

Abstract

The present study was aimed to assess the microbiological quality of street vended drinking water of Dhaka city. The water samples were collected from street vendors in different areas of Dhaka city. All of the 30 samples were found having microorganisms higher than WHO limits for drinking water. Four (13.34%) samples were confirmed to have *Salmonella* contamination and twenty (66.67%) samples were contaminated with *Pseudomonas*. Based on morphological and biochemical characterization *Salmonella* isolates were identified as *Salmonella choleraesuis* and *Salmonella bongori*. Among the isolates of *Pseudomonas*, fourteen were identified as *Pseudomonas alcaligenes* and six were as *Pseudomonas aeruginosa*. During antibiogram for *Salmonella*, 100% of the isolates were found resistant to Penicillin. Chloramphenicol, doxycycline, Gentamycin, Neomycin were sensitive to all of the isolates. The *Pseudomonas* isolates showed a significant drug resistance to Penicillin (100%), Ampicillin (95%), Amoxicillin (95%) and Nalidixic acid (85%). The present study demonstrates that drinking water samples from street vendors in Dhaka city are not complying with microbiological specifications of WHO and indicates that street vending drinking water in Dhaka city may not be safe for human consumption and also shows that these are the potential sources of drug resistance *Salmonella* and *Pseudomonas*.

Keywords: Street vended drinking water; Antibiotic resistance; *Salmonella*; *Pseudomonas*.

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doi: [http://dx.doi.org/10.3329/jsr.v6i2.17640](http://dx.doi.org/10.3329/jsr.v6i2.17640)


1. Introduction

The quality of drinking water is of vital concern to mankind, since it is directly associated with human life [1]. Water is unsafe for human consumption when it contains pathogenic or disease-causing microorganisms, chemicals, heavy metals etc. Food and water-borne pathogens such as *Salmonella, Campylobacter*, and *Escherichia coli* and recently *Listeria*

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monocytogenes pose a major health threat globally [2]. A global estimation by World Health Organization (WHO) indicated that in 2005, nearly 1.8 million people died from diarrhoeal disease primarily due to the consumption of contaminated food and drinking water. Although most Salmonella infections in human are self-limited confine to gastrointestinal tract, but when infection spreads beyond the intestine causing severe infections that spread to the bloodstream, meningeal linings of the brain or other deep tissue, it requires an appropriate therapeutic intervention with antibiotics. In the recent times, another major concern is the occurrence of multi drug resistance among the Salmonella [3]. It is presumed that the extensive use of antibiotics, especially in livestock production, may have resulted in the increasing incidence of antibiotics resistance in food borne Salmonella [4]. Pseudomonas species are Gram negative motile rods belonging to the family Pseudomonaceae and found in various environments including water, soil and rhizosphere [5]. Pseudomonas is a clinically significant and opportunistic pathogen, often causing nosocomial infections, particularly among immune compromised patients. They induce a variety of human infections, including bacteremia, respiratory infections, genitourinary tract infections, and wound infections [6]. One of the most worrying characteristics of Pseudomonas is its low antibiotic susceptibility. Pseudomonas easily develops resistance either by mutation in chromosomally-encoded genes, or by the horizontal gene transfer of antibiotic resistance determinants [7]. Antibiotic resistance by bacteria has been recognized as a major medical problem facing humankind [8]. The selection of effective antibiotics is critical for the treatment of invasive infections, but has become more difficult as antibiotic-resistance has increased [9]. The occurrence and spread of antibiotic resistant bacteria (ARB) are pressing public health problem worldwide and they are available in water. This study was conducted to evaluate the microbiological quality of street vending drinking water and identification of different species of Salmonella and Pseudomonas and characterization of antibiotic susceptibility of the isolates from water samples in Dhaka city.

2. Material and Methods

2.1. Sample collection

A total of 30 filter drinking water samples were collected from street vendors (commonly known as filter water) from 10 different areas [Mohammadpur (M), Shahabag (SB), Dhanmondi (DM), New Market (NM), Farmgate (FG), Gulistan (Gu), Kawran Bazar (KB), Mirpur (MP), Mohakhali (MK), Gulshan (G)] in Dhaka city, Bangladesh. Sterile Schott Duran bottles were used to collect the samples aseptically. All the samples were kept immediately at 4°C analyzed accordingly.

2.2. Microbiological quality analysis of water

Enumeration of Aerobic Plate Count (APC), Enterobacteriaceae and E. coli were performed according to ISO standard method (ISO-4833:2003(E), Microbiology of food
and animal feeding stuffs-Horizontal method for the enumeration of microorganisms-Colony-count technique at 30°C). Enumeration of microorganisms was done by serial dilution technique followed by pour plate technique and plate count agar was used. Enumeration of Enterobacteriaceae and *E. coli* was performed by Most Probable Number (MPN) method. For presumptive test of Enterobacteriaceae and *E. coli* sterile LST broth was used. EE and EC broth were used for the confirmation of Enterobacteriaceae and *E. coli*.

2.3.1. *Enrichment of salmonella*

The samples were inoculated into Buffer peptone water for pre-enrichment and incubated at 35-37°C for 24±2 hrs. For selective enrichment the cultures from the pre-enrichment media were inoculated into Tetrathionate Broth, contents were mixed well and incubated at 35-37°C for 24±2 hrs.

2.3.2 *Isolation of salmonella*

Vortex-mixed samples from Tetrathionate broths were streaked onto the surface of Xylose Lysine Deoxycholate (XLD) agar, Salmonella Shigella (SS) agar and Bismuth Sulfite Agar (BSA) using sterile inoculating loops and then the dishes were covered, inverted and incubated at 35-37°C for 24±2 hrs. The presumptive *Salmonella* colonies were then sub-cultured by streaking onto the fresh XLD agar, SS agar, and BSA using a sterile inoculating loop and then incubated at 35-37°C for 24±2 hours for obtaining only single type of colonies. After incubation, typical or suspicious *Salmonella* colonies were examined.

2.3.3. *Confirmation of the salmonella isolates*

The presumptive *Salmonella* isolates were identified by three confirmatory biochemical tests, Triple Sugar Iron (TSI) agar test, Lysine Iron Agar (LIA) test and the urease test. Two or more suspicious colonies were selected from each XLD, SS and BSA plate. TSI slant was inoculated with a portion of each colony by streaking slant and stabbing butt, after inoculating TSI slant, LIA slant was also inoculated by stabbing butt without flaming, and TSI, LIA was incubated with loosened caps at 35-37 °C for 24±2 hrs. All presumptive *Salmonella* from TSI and LIA slant were tested for urease production. For the urease test, 2 loopful of pure and well isolated Salmonella colonies were inoculated into the urea broth and incubated with loosened caps at 35 °C for 48 h. Only urease negative cultures were *Salmonella* which were isolated for further biochemical characterization.

2.4.1. *Enrichment of pseudomonas*

The samples collected were inoculated into buffer peptone water for enrichment and incubated at 35-37°C for 24±2 hrs.
2.4.2. Isolation of pseudomonas

From enrichment media, one (1) loop full was streaked on Cetrimide agar and Pseudomonas agar containing petri-dishes. Then the plates were kept at 35-37 °C for 24-48 hrs. After incubation, the plates were observed for greenish fluorescence colony.

2.4.3. Confirmation of the pseudomonas isolates

Oxidase test: A filter paper (Whatman No. 1) was taken and 2-3 drops of 1% solution of N,N,N’,N’- tetra methyl 1-p-phenylendiamine dihydrochloride on filter paper. By loop, suspected colony was smeared on it. Purple color was produced within 5-10 seconds. Oxidase positive colonies were presumptive *Pseudomonas* and isolated for biochemical characterization.

2.5. Maintenance and preservation of the isolates

Presumptive colonies were transferred to nutrient agar slants and two slants of each isolate were kept in refrigerator at 4 °C for further study. Occasional sub culturing (3/4 weeks) was maintained to keep the cultures in active condition characters unimpaired.

2.6. Identification of different species of salmonella and pseudomonas

The isolated colonies were identified on the basis of morphology, cultural characters and their biochemical profile. Arrangement of vegetative cells, shape and gram reaction were observed under microscope after proper straining of the isolates. The test organisms were stained by Gram’s Method to determine their staining characteristics. Biochemical test like Urease production test, Oxidase test, Catalase reaction, Indole test, Citrate utilization test, Production of hydrogen sulphide, Methyl-Red (MR) test, Voges-proskauer (V.P.) test, Fermentation test, Lysine decarboxylase and motility test, Nitrate reduction, Gelatin liquefaction, Starch hydrolysis were done for identification of different species of *Salmonella* and *Pseudomonas*.

2.7. Antimicrobial susceptibility testing

The antimicrobial susceptibility testing was done by the agar disk diffusion method as described by Clinical and Laboratory Standards Institute (CLSI) [10]. The isolates were tested for susceptibility to Amoxycillin 10µg, Ampicillin 30 µg, Chloramphenicol 30 µg, Doxycycline 30 µg, Erythromycin 15 µg, Kanamycin 30 µg, Nalidixic acid 30 µg, gentamicin10 µg, Neomycin 30 µg, and Penicillin 10 µg on Muller-Histon agar plates. Pure colonies of isolated *Salmonella* and *Pseudomonas* were emulsified in normal saline and turbidity was matched with 0.5 McFarland turbidity standards. The respective antibiotics discs were placed on the culture plates. The plates were incubated at 35 °C for 24 h and inhibition zone were measured. The sensitivity and resistance of the isolates towards the antibiotics were determined as per the CLSI.
3. Results and Discussion

3.1. Enumeration of viable bacterial counts

A total of 30 water samples from street vendors were collected from 10 areas in Dhaka city. Three parameters viz. Aerobic Plate Count, *E. coli* and Enterobacteriaceae were studied for the microbiological quality analysis of water. The result of aerobic plate counts and enumeration of *E. coli* and Enterobacteriaceae are documented in (Table 1).

Table 1. Result of enumeration of microorganisms (APC), Enterobacteriaceae, *E. coli*, *Salmonella* and *Pseudomonas*.

<table>
<thead>
<tr>
<th>Sample ID</th>
<th>APC (cfu/ml)</th>
<th>Enterobacteriaceae (MPN/100ml)</th>
<th><em>E. coli</em> (MPN/100ml)</th>
<th><em>Salmonella</em></th>
<th><em>Pseudomonas</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>M1</td>
<td>$4 \times 10^3$</td>
<td>17</td>
<td>0</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>M2</td>
<td>$2.3 \times 10^4$</td>
<td>22</td>
<td>4.5</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>M3</td>
<td>$2.1 \times 10^4$</td>
<td>1600</td>
<td>28</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>SB1</td>
<td>$1.75 \times 10^5$</td>
<td>47</td>
<td>47</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>SB2</td>
<td>$1.65 \times 10^5$</td>
<td>1600</td>
<td>54</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>SB3</td>
<td>$1.35 \times 10^5$</td>
<td>240</td>
<td>49</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>DM1</td>
<td>$2.35 \times 10^5$</td>
<td>39</td>
<td>6.1</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>DM2</td>
<td>$6.25 \times 10^5$</td>
<td>39</td>
<td>14</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>DM3</td>
<td>$1.11 \times 10^5$</td>
<td>280</td>
<td>28</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>NM1</td>
<td>$4.4 \times 10^4$</td>
<td>28</td>
<td>140</td>
<td>Present</td>
<td>Absent</td>
</tr>
<tr>
<td>NM2</td>
<td>$9.7 \times 10^3$</td>
<td>140</td>
<td>9.2</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>NM3</td>
<td>$5.5 \times 10^4$</td>
<td>54</td>
<td>14</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>FG1</td>
<td>$3.2 \times 10^5$</td>
<td>$&gt;1600$</td>
<td>47</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>FG2</td>
<td>$4.3 \times 10^5$</td>
<td>$&gt;1600$</td>
<td>20</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>FG3</td>
<td>$2 \times 10^4$</td>
<td>0</td>
<td>4</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Gu1</td>
<td>$2.3 \times 10^3$</td>
<td>22</td>
<td>17</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Gu2</td>
<td>$3.4 \times 10^3$</td>
<td>6.8</td>
<td>14</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>Gu3</td>
<td>$4.2 \times 10^4$</td>
<td>14</td>
<td>22</td>
<td>Absent</td>
<td>Absent</td>
</tr>
<tr>
<td>KB1</td>
<td>500</td>
<td>22</td>
<td>17</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>KB2</td>
<td>80</td>
<td>6.8</td>
<td>14</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>KB3</td>
<td>350</td>
<td>14</td>
<td>22</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>MP1</td>
<td>$5.1 \times 10^4$</td>
<td>0</td>
<td>0</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>MP2</td>
<td>$1.5 \times 10^5$</td>
<td>0</td>
<td>2</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>MP3</td>
<td>$2.35 \times 10^5$</td>
<td>0</td>
<td>0</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>MK1</td>
<td>$2.5 \times 10^5$</td>
<td>7.8</td>
<td>4.5</td>
<td>Present</td>
<td>Present</td>
</tr>
<tr>
<td>MK2</td>
<td>$2.4 \times 10^5$</td>
<td>21</td>
<td>7.8</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>MK3</td>
<td>$2 \times 10^5$</td>
<td>350</td>
<td>$&gt;1600$</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>G1</td>
<td>$3.65 \times 10^3$</td>
<td>49</td>
<td>11</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>G2</td>
<td>$4.4 \times 10^5$</td>
<td>17</td>
<td>0</td>
<td>Absent</td>
<td>Present</td>
</tr>
<tr>
<td>G3</td>
<td>$3.75 \times 10^5$</td>
<td>110</td>
<td>0</td>
<td>Absent</td>
<td>Present</td>
</tr>
</tbody>
</table>
According to WHO (2011) Guideline for Drinking Water Quality the standard for APC is $1 \times 10^3$ cfu/mL and 0 (MPN)/ml for *E. coli*. The Aerobic Plate Count in water sample was found range from 80 to $4.4 \times 10^5$ cfu/ml. *E. coli* and Enterobacteriaceae count was ranged from 0 to $>1600$ (MPN)/100 ml. 4 samples were found contaminated with *Salmonella* and 20 samples were contaminated with *Pseudomonas*. Table 1 shows that 86.67% of the water samples exceeded WHO guideline for APC, 83.34% of the water sample exceeded WHO guideline for *E. coli* and all the 30 samples exceeded WHO guideline for drinking water for any one of the parameter. These values for APC and *E. coli* were found unacceptable for drinking water [11]. Mahbub et al. [12] reported among 45 samples, collected from different outlets of WASA water supply chain, 26 samples exceed the WHO standard for APC and 57.78% samples exceed for coliform and 51.11% for *E. coli* bacteria. Vagarali et al. [13] reported that in India out of 30 samples 10 samples (33.33%) were contaminated with either one or more than one type of organisms. It is well known that quality and safety of drinking water constitutes to be an important public health issue, because its contamination is responsible for the transmission of infectious disease that cause serious illness. International water-quality standards permit no detectable level of harmful pathogen in drinking water. Data from present study demonstrate that drinking water samples from street vendors in Dhaka city are not complying microbiological specifications of WHO and indicate that street vending drinking water in Dhaka city may not be safe for human consumption.

### 3.2.1. Isolation of salmonella

Out of 30 water samples a total of 10 samples were found positive for *Salmonella* based on growth characteristics on three selective media (BSA, XLD and SS agar). Out of 10 suspected *Salmonella* isolates only 4 isolates (SM1, SNM1, SFG1, SMK1) were confirmed by biochemical studies such as TSI, LIA and urea hydrolysis test. The presumptive colonies were isolated as *Salmonella* if the colonies showed alkaline slant and acidic butt on TSI agar, purple slant on LIA and did not hydrolyze Urea.

### 3.2.2. Biochemical characterization of salmonella isolates

All the isolates were found Gram negative, short rod under microscope after proper straining of the isolates which correspond to the morphological characteristics of *Salmonella*. Specific biochemical and carbohydrates fermentation studies were also carried out to identify different species of *Salmonella*. The results of the biochemical test are shown in Table 2.
Table 2. Biochemical characteristics of the *Salmonella spp* isolated from drinking water.

<table>
<thead>
<tr>
<th>Test parameters</th>
<th>Biochemical test results</th>
<th>S M1</th>
<th>S NM1</th>
<th>S FG1</th>
<th>S MK1</th>
</tr>
</thead>
<tbody>
<tr>
<td>TSI (red slant &amp; yellow but)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>LIA (purple slant)</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\text{H}_2\text{S}$ from TSI slant</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Lysin decarboxylase</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Catalase test</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Citrate utilization</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Oxidase test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>MR test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>VP test</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Motality</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Indole</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Urease</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Gram staining</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Arabinose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Xylose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Glucose</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Lactose</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Rhamnose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
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<tr>
<td>Melibiose</td>
<td>-</td>
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<td>+</td>
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<tr>
<td>Sorbitol</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Trehalose</td>
<td>-</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Note: ‘+’ indicates positive reaction and ‘–’ indicates negative reaction.

3.2.3. Presumptive isolation of salmonella isolates

All of the 4 isolates were motile and fermented glucose but did not ferment lactose which is special biochemical character of *Salmonella spp*. The above characteristics (Table 2) indicate that the isolates belong to the genus *Salmonella* and found closely related to the species of *Salmonella choleraesuis* (isolates SM1, SMK1) and *Salmonella bongori* (isolates S NM1, S FG1) while compared with the standard description given in Bergey’s Manual of Determinative Bacteriology [14]. In this investigation out of 30 samples 4 samples were found positive for *Salmonella* and the total percentages of occurrence was 13.34%. These results agree with Bhatta et al., who reported that out of 300 samples only 14% were reported to be positive for *Salmonella* and the organisms were identified as *S. typhi*, *S. paratyphi A*, *S. typhimurium* and *S. enteritidis* [15]. In Nepal, Shrestha et al. [16] reported 4.7% occurrence of *Salmonella*, of which 1 (10%) was *S. paratyphi A* and 9 (90%) were non-typhi, in 86 water samples collected from urban water supply system of Kathmandu and Rasheed et al. [17] reported that 5% of the drinking water samples
collected from Lahore, Sargodha and Sahiwal were contaminated with *Salmonella* which is lower than our present study.

### 3.2.4. Antimicrobial resistance patterns of salmonella isolates

The antibiotic sensitivity assay of the *Salmonella* isolates against commercial antibiotics illustrated that 100% of the *Salmonella* isolates were found resistant to Penicillin followed by Amoxicillin (75%), Ampicillin (50%) and in the case of Erythromycin, Nalidixic acid, Kanamycin, only one isolate (25%) was resistant, whereas the rest of the isolates were either susceptible or intermediate. 75% of the isolates were intermediately susceptible to Erythromycin. With reference to Chloramphenicol, doxycycline, Gentamycin, Neomycin, all of the isolates were sensitive. Nalidixic Acid and Kanamycin were sensitive to 75% of the isolates followed by Ampicillin (50%). This indicates that the Penicillin, Amoxicillin and Ampicillin can hardly be considered for the treatment of Salmonella infections as compared to Chloramphenicol, doxycycline, Gentamycin, Neomycin. Besides, 25% of the isolates showed multiple-drug-resistance (MDR) to five antibiotics, while, the rest 75% of the isolates exhibited MDR to 2 antibiotics. The percentage of resistance is presented in Fig. 1. Ahmed *et al*. [18] reported that about 87.5% isolates were found resistant to amoxicillin and ampicillin followed by erythromycin (62.5%), doxycycline (50%) which is higher than our present study. While Chloramphenicol sensitivity was 100% in all the isolates. Kanamycin and doxycycline was sensitive in 50% of the isolates [18]. In Malaysia Gunasegaran *et al*. [19] reported that all (100%) of the *Salmonella* isolates were resistant to ampicillin, tetracycline and chloramphenicol. In case of penicillin only 28.6% isolates were resistant and all the isolates were susceptible to kanamycin. They also examined that 71.4% of the isolates showed MDR to three antibiotics and rest 28.6% of the isolates exhibited MRD to four antibiotics [19].

![Graphical representation of percentage of *Salmonella* isolates show their response to antibiotics used in the study.](image-url)
Comparable observations were also reported by Mafu et al. [20] who demonstrated the 90% of the isolates from domestic and waste water samples were susceptible to neomycin and kanamycin while 10% were intermediate. Against doxycycline, 57.5% of isolates were resistant. In Nepal, Shrestha et al. [21] reported that all the Salmonella isolates were 100% susceptible to chloramphenicol, nalidixic acid and 70% were resistant to amoxicillin. White et al. [22] examined that most of the Salmonella isolates were susceptible to antimicrobials tested. Twenty-eight (36%) Salmonella isolates were resistant to at least one antimicrobial and 10 (13%) isolates displayed resistance to four or more antimicrobials.

3.3.1. Isolation of pseudomonas

Out of 30 water samples 20 samples were found Pseudomonas positive by appropriate microbiological methods and biochemical tests. The presumptive Pseudomonas isolates were confirmed by biochemical tests such as oxidase test, Gram staining and Catalase test. All of the 20 isolates of suspected Pseudomonas were found positive for Pseudomonas oxidase test, Gram staining and Catalase test. The pure colonies displayed Greenish fluorescence on Pseudomonas Agar (PA) and Cetrimide Agar (CA).

3.3.2. Identification of different species of pseudomonas

The isolated colonies were identified on the basis of morphology, cultural characters and their biochemical profile. In Gram staining the morphology of the isolated bacteria was rod shape, gram negative, single or paired in arrangement which was similar with morphological characters of Pseudomonas. Specific biochemical and carbohydrates fermentation studies were carried out to identify different species of Pseudomonas. The results of the biochemical test are shown in the (Table 3). Based on the above biochemical characteristics (Table 3), 14 out of 20 isolates (PM1, PM2, PM3, PFG3, PKB1, PKB2, PKB3, PMP2, PMP3, PKB1,PKB3, PFG1, PFG2, PG1, PG2, PG3) found closely related to the species of Pseudomonas alcaligens and rest 6 isolates (PFG1, PGu1, PGu2, PGu3, PMP1, PMK2) were identified as Pseudomonas aeruginosa while compared with the standard description given in “Bergey’s Manual of Determinative Bacteriology”. In this investigation 20 out of 30 samples were found positive which correspond 66.67% of occurrence. The occurrence of Pseudomonas was higher than some literature cited. In India, Vagarali et al. [23] reported 20% prevalence of Pseudomonas in drinking water sample.
Table 3. Biochemical characteristics of the *Pseudomonas spp* isolated from drinking water.

<table>
<thead>
<tr>
<th>Test parameters</th>
<th>Isolates Identified and their Biochemical Behavior</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>pseudomonas</em> P. <em>aeruginosa</em> P. <em>aeruginosa</em> P. <em>aeruginosa</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>aeruginosa</em> P. <em>alcaligenes</em> P. <em>aeruginosa</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em> P. <em>alcaligenes</em></td>
</tr>
</tbody>
</table>

- **Citrate**
  - PM1: +
  - PM2: -
  - PM3: -
  - PG1: +
  - PG2: -
  - PG3: -

- **Oxidase**
  - PM1: +
  - PM2: +
  - PM3: +
  - PG1: +
  - PG2: +
  - PG3: +

- **MR**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: +
  - PG2: +
  - PG3: +

- **VP**
  - PM1: +
  - PM2: +
  - PM3: +
  - PG1: +
  - PG2: +
  - PG3: +

- **Mortality**
  - PM1: +
  - PM2: +
  - PM3: +
  - PG1: +
  - PG2: +
  - PG3: +

- **Indole**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: -
  - PG2: -
  - PG3: -

- **Urease**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: -
  - PG2: -
  - PG3: -

- **Growth at 50°C**
  - PM1: +
  - PM2: +
  - PM3: +
  - PG1: +
  - PG2: +
  - PG3: +

- **Growth at 37°C**
  - PM1: +
  - PM2: +
  - PM3: +
  - PG1: +
  - PG2: +
  - PG3: +

- **Nitrate reduction**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: -
  - PG2: -
  - PG3: -

- **Gelatin liquefaction**
  - PM1: +
  - PM2: +
  - PM3: +
  - PG1: +
  - PG2: +
  - PG3: +

- **Starch hydrolysis**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: -
  - PG2: -
  - PG3: -

- **Glucose, acid**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: -
  - PG2: -
  - PG3: -

- **Lactose, acid**
  - PM1: -
  - PM2: -
  - PM3: -
  - PG1: -
  - PG2: -
  - PG3: -

Note: ‘+’ indicates positive reaction and ‘–’ indicates negative reaction.

3.3.3. *Antibiotic resistance patterns of the isolated pseudomonas*

The antibiotic sensitivity assay of the *Pseudomonas* isolates against commercial antibiotics showed that 100% of the *Pseudomonas* isolates were found resistant to Penicillin followed by Ampicillin (95%), Amoxicillin (95%), Nalidixic acid (85%), Erythromycin (70%), and Doxycycline (30%). 45% of the isolates were found susceptible to Kanamycin followed by Chloramphenicol (35%). With reference to Gentamycin and Neomycin, all of the isolates were sensitive to these antibiotics. Chloramphenicol was sensitive to 65% of the isolates followed by Doxycycline (60%), Kanamycin (35%), Erythromycin (30%), Nalidixic acid (10%), Ampicillin and Amoxicillin (5%). Besides, 40% of the isolates showed multiple-drug-resistance (MDR) to five antibiotics, 25% of the isolates exhibited MDR to six antibiotics. The percentage of resistance pattern is presented in Fig. 2. Igbinsosa et al. [24] reported that all isolates (100%) from fresh water and mixed liquor samples from Alice were susceptible to ciprofloxacin and gentamicin. Conversely, all (100%) were resistant to penicillin.
safe for human consumption. These water sources also harbor pathogens which were found contaminated with some literature cited. Hirulkar and Soni reported that out of 44 water samples 22 samples were found contaminated with *Pseudomonas*. All 22 isolates showed maximum resistance to Erythromycin (50%). Some antibiotic like Ampicillin (41%), Penicillin (41%) and Amoxycillin (41%) were less effective or minimum resistances against the isolates [25].

The present study indicates the occurrence *Salmonella* and *Pseudomonas* in the water samples. This study has also confirmed the prevalence of a varying drug resistance pattern among the *Salmonella* and *Pseudomonas* isolates. This may be due to the presence of more than one serovar of *salmonella* and *Pseudomonas* in the water sample. The occurrence and spread of antibiotic-resistant bacteria (ARB) are pressing public health problems worldwide. Proper use of antibiotic protects our life but uncontrolled and irresponsible use of antibiotics is responsible for the occurrence of the antibiotic resistant strains among the pathogens. Increasing antibiotic resistance can limit the therapeutic options available to physicians for clinical cases that require antibiotics treatment. Therefore, there is a need to find strategies to minimize the risk of spreading antimicrobial resistance among animal and human populations.

4. Conclusion

The quality of drinking water is of vital concern to mankind, since it is directly associated with human life. The microbial contamination of drinking water and its control constitutes a major issue worldwide because it is still a major source of infection and can cause mortality especially in the children and threatens the health of the population both in developed and developing regions. Our present study suggests that, drinking water from street vendors in Dhaka city are contaminated with a large group of bacteria and are not safe for human consumption. These water sources also harbor pathogens which are
causative agents of fatal diseases. The *Pseudomonas* and *Salmonella* isolated from water samples showed resistance to most of the antibiotics tested. Their multi-drug resistance pattern is a matter of great concern since these bacteria may no longer be treated with conventional therapeutic drugs and they are also capable of spreading their resistance gene to other bacterial genera. In view of this research there is a need to control of quality of street vending drinking water in Dhaka city for complying BDS and WHO standards for APC and *E. coli* counts and need to develop good and hygienic water treatment process to prevent occurrence of drug resistance *Salmonella* and *Pseudomonas* in drinking water.

**Acknowledgments**

The authors would like to thank the Department of Food Engineering & Tea Technology, Shahjalal University of Science & Technology, Sylhet-3114, Bangladesh and Institute of Food Science & Technology, Bangladesh Council of Scientific & Industrial Research, Dhaka-1205, Bangladesh for supporting this research project.

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