Microbial safety of drinking jar water and antimicrobial resistant pattern against *Escherichia coli* in jar water at Chittagong, Bangladesh

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**Abstract**

Opportunist pathogens *Escherichia coli* and *Salmonella* spp. enter into human body through consumption of drinking water, causes different gastrointestinal disorders like diarrhea, dysentery and water borne infections like cholera, typhoid etc. This study was carried out to determine the microbial safety of drinking jar water and antimicrobial resistant pattern against *Escherichia coli* in jar water samples at Chittagong, Bangladesh. About 189 drinking jar water samples were collected from 63 water treatment plants and market store over a period of two months. Most probable number (MPN) test was done to detect the coliforms in drinking water samples. The MPN number was high (63) after 24 hours incubation. The test results showed that 41 (21%) water samples has presence of coliforms and 27 (14%) plant water samples contain *E. coli*. But unfortunately *Salmonella* Spp. was not found in any of the samples. Conformation test of *E. coli* were performed by Polymeric Chain Reaction (PCR). The *E. coli* isolates were susceptible to Enrofloxacin (100%), Cephradine (100%), and Gentamycin (92.59%) with an intermediate sensitivity to Amoxicillin (7.40%) and Ceftriaxone (3.70%). The organisms showed 81.48%, 74.02% and 70.30% resistance to Ampicillin, Colistinsulphate, and amoxicillin respectively. So bacteriological assessment of all water sources for drinking should be planned and conducted on regular basis for considering the public health significance of these zoonotic pathogen that are transmitted through drinking jar water.

**Introduction**

Water is an indispensable commodity in human life, which should be easily accessible, adequate in quantity, free of contamination safe, affordable, and available throughout the year in order to sustain life (Al-Khatib et al., 2003). Promoting safe drinking water in developing countries is implied due to the persistently high levels of water related morbidity and mortality. When unsafe drinking water coupled with poor sanitation, it globally kill at least 1.6 million children under the age of five every year, 84% of them living in rural areas. If the current trend persists, within 2015 nearly 1.7 billion rural dwellers will not have access to safe water and improved sanitation (WHO, 2003). Availability of water implies sufficient quantity and good quality. Adequate supply of quality water is essential to maintain good public and community health since protection of water resources from contamination is the first priority (Daud et al., 2001). The contaminated water or inadequate supply of safe drinking water causes different gastrointestinal disorders like diarrhea, dysentery and water borne infections like cholera, typhoid etc. It is presently clear that the greater part of the enteric disorders of human and animal are transmitted through contaminated food and water (Johnson et al., 2003). Coliform bacteria have been used for many years to determine the quality and safety of water for human consumption. Escherichia coli and other groups of coliforms may be present where there has been fecal contamination originating from warm-blooded animals (Chao et al., 2003). Water is a universal solvent it dissolves...
salts, inorganic and organic compounds and gases that take part in metabolic reactions, maintain the macromolecular framework, stabilize plasma membrane, thermoregulation, transport nutrients, and maintain homeostasis and body volume/weight (Armstrong et al., 2007). As per microbiological criteria, water quality is characterized by the event of fecal pointers, that is, Escherichia coli and Salmonella spp. In addition to indicating the potential presence of pathogens, these fecal microorganisms raise the issue of the spread of anti-microbial resistance, a major health concern today. Some authors consider water to be the main link between the four major ecosystems – human, animal, soil, and aquatic – involved in the circulation of antibiotic resistance (Nwosu, 2008). From the view of public health, it is highly appreciated that potable water supply system should be safe. Water may be polluted at its sources by excreta or sewage, which is almost certain to have pathogenic microorganisms. Potable water system can become polluted with coliform and pathogenic bacteria from normal diseased or carrier human and animal excrement. As a result, microbiological examination of water should routinely be carried out to monitor and control the quality and safety of drinking water. Although substantial amount of work has been carried out in Bangladesh, unfortunately a little information is available in the published format. Therefore, the present study was conducted to determine the microbial safety of drinking jar water and antimicrobial resistant pattern against Escherichia coli in those jar water samples available in Chittagong area.

Materials and Methods

Sample collection

The study was set up to determine the microbial safety of drinking jar water and antimicrobial resistant pattern against Escherichia coli in jar water samples at Chittagong, Bangladesh. Chittagong is the second largest city and different classes of people are living here. There are so many water treatment plants whose produce jar water is present here. So this place was selected without any hesitation that will provide maximum information, convenient to collect and analyze the sample. About 189 drinking jar water samples were collected from 63 water treatment plants and from market. Water samples were collected in 50 ml capacity sterile bottles. Care in taken before collecting samples from the jar in order to prevent any contamination in bottles. After collecting the water from jar, the caps of the bottles were closed tightly. Three or four samples were wrapped by aluminum foil paper and finally transferred to the laboratory. Samples were stored at 4°C temperature in the laboratory prior to testing (Ukhun et al., 2005). The study was carried out during the period from November, 2015 to April, 2016.

MPN count

Most Probable Number (MPN) method is a statistical, multi-step assay consisting presumptive, confirmed and completed phases for water examination for the presence of coliforms. An estimate of the number of coliforms (MPN) can also be done in the presumptive test. Most Probable Number (MPN) test was conducted by 9 test tube method. At first 9 sterile screws capped test tubes with Durham’s tube inside (3 large and 6 small) were autoclaved with 10 ml MacConkey broth. After autoclaving these test tubes were divided into three groups and then 10 ml, 1 ml and 0.1 ml of sample was inoculated in each tube of three groups. Finally they were incubated at 37°C for 24 hr. After 24 hr color changing and gas formation were observed and the number of coliforms were counted by MPN chart (Oblinger and Koburger, 1975).

Isolation of E. coli and Salmonella by nutrient agar

Sterilized platinum loop was used for streaking the MacConkey broth on Nutrient Agar to get pure culture of E. coli and Salmonella. All inoculated media were kept at 37°C for overnight in an incubator. E. coli and Salmonella were grown here after incubation.

Sub-culturing of E. coli and Salmonella in MacConkey agar

Sterilized platinum loop was used for streaking the inoculums from Nutrient Agar on MacConkey agar for more enrichment of E. coli and Salmonella. All inoculated media were kept at 37°C for overnight in an incubator.

Isolation of E. coli

Sterilized platinum loop was used for streaking the inoculums from MacConkey Agar on EMB agar, to get isolates of pure culture. All inoculated media were kept at 37°C for overnight in an incubator. After incubation showed smooth circular colonies with dark centers and metallic sheen indicate the positive result for E. coli.

Identification of E. coli isolates by Indole test

For Indole test, a strip of filter paper was soaked in oxalic acid and dried and then hung in the form of loop over the Tryptone broth in a culture tube. The
ends of the paper was securing between the cotton plug and mouth of the tube. The development of red color in the paper was regarded as positive for Indole. Tryptone broth culture was incubated at 37°C for 24 hours. _E. coli_ will show positive Indole production.

**Molecular confirmation of _E. coli_ by PCR**

In order to develop a quick and reliable confirmatory diagnostic test using molecular method PCR was tried for the presence of _E. coli_. For molecular detection all the culturally and biochemically positive samples (27) were selected for DNA extraction. PCR amplification of specific DNA fragments was then conducted using specific primer sets (targeting 16S rRNA gene) for _E. coli_. The PCR products were then run in 1% agarose gel with ethidium bromide, incorporating 100 bp size markers and visualized under UV light. All positive _E. coli_ (cultural and Biochemical test) isolates produced expected bands at 585 bp regions confirming the isolates to be _E. coli_. The results obtained from PCR assay for _E. coli_ is observed in gel electrophoresis.

**Isolation of _Salmonella_ spp.**

Sterilized platinum loop was used for streaking the inoculums from MacConkey agar on BGA agar, to get isolates of pure culture. All inoculated media were kept at 37°C for overnight in an incubator.

**Identification of _Salmonella_ spp. by XLD agar**

For more conformation of identified _Salmonella_ from BGA agar, bacterial inoculums from BGA agar were further streaking on XLD agar and incubated with the same way. After incubation black centered colony of _Salmonella_ was not found that indicate negative test for _Salmonella_.

**Identification of _Salmonella_ spp. by TSI slant**

A straight inoculating needle was used to pick up isolated colony from Nutrient Agar. The TSI slant was inoculated by stabbing the butt down to the bottom, and then streaked over the surface of the slant. The TSI slant was then incubated overnight at temperature of 37°C. After incubation black coloration was not found. So this test indicates the negative result for _Salmonella_.

**Identification of _Salmonella_ by stereotyping**

The polyvalent agglutinating antiserum poly “O” and poly A-I” against _Salmonella_ manufactured by S and A Reagents Lab, Bangkok, Thailand, was used for the stereotyping of the isolated _Salmonella_. The macroscopic slide agglutination tests were performed. The cultures to be tested were first checked with salmonella poly “O” polyvalent antiserum. A single isolated colony from BG agar was dissolved in physiological saline solution. One drop of thick bacterial suspension was placed on glass slide and a drop of polyvalent antiserum was added. The slide was gently rotated to mix the contents thoroughly. Those cultures that agglutinated within one to two minutes were selected as positive for _Salmonella_ and subjected to agglutination test with _Salmonella_ agglutinating antiserum (poly “A-I”).

**CS test at Muller Hinton agar**

After confirmation of isolates as _E. coli_, antimicrobial susceptibility of the isolates were determined by using the micro disc diffusion method, and the method was used according to guidelines established by Clinical and Laboratory Standards Institute (CLSI), 2010.

**Results**

The results of isolation and identification of _E. coli_ and _Salmonella_ spp. in MPN count, Nutrient agar, MacConkey agar, XLD agar, Indole test, TSI slant, PCR and Stereotyping were given in Table 1. The sample that gives bright pink colonies on MacConkey agar due to fermentation of lactose, selected as positive to coliforms. Then only MacConkey positive samples were again sub-cultured on EMB agar and which produce metallic sheen color due to the precipitation of methylene blue in the medium and the very high amount of acid produced from lactose fermentation represent positive test for _E. coli_. For more conformation TSI slant test and PCR also done and they also represent positive result for _E. coli_. Result of PCR for 16s rRNA gene of _E. coli_ is shown in Figure 1.

For isolation and identification of salmonella several types of cultural and biochemical test were done. Here only positive samples of MacConkey agar were sub-cultured on BGA, XLD agar and suspected colony characteristics were used as inoculums for further confirmation in TSI slant and stereotyping was also performed by _Salmonella_ polyvalent antisera. But negative results were observed in all cases.

Table 2 Antibiotic resistance in _E. coli_ strains isolated from water samples. Resistant to Ampicillin and Colistin sulfate was found in 22 (81.41%) and 20 (74.02%) samples respectively. Both amoxicillin and ceftriaxone was shown resistant in 19 (70.30%) samples. Gentamcin was resistant in 2 (7.42%) samples, whereas no resistance 0 (0%) was shown against Enrofloxacin and Cephradin.
Discussion

Total faecal coliforms have traditionally been regarded as indicators of microbial contamination of waters and *E. coli* is the best indicator for assessment of fecal contamination (Edberg et al., 1998). According to the WHO recommended limits for potable water, the permissible limit should not exceed one coli forms colony-forming unit per 100 ml water; and should contain no colony-forming unit of *E. coli* type 1 per 100 ml water (Cheesbrough, 2000). The present study shows 69% samples contain coliforms and among these 43.55% samples contain *E. coli*. Compare to other study in different parts of Bangladesh the percentage is less in number. It is also assume that in Chittagong, water layer up to 400 feet’s mostly contain *E. coli* but most of the Jar Water treatment plant raised water below this point. This information is gathered by conversation with several engineer’s and plant employees. The sale of polluted water in containers in different parts of the country has turned into a booming business for lack of monitoring and control by the authorities concerned posing a serious health hazard to consumers. The results of this study reveal that average bacterial density in drinking water was relatively high, especially from unprotected water sources, compared with that from protected sources. The presence of *E. coli* in water suggests enteric pathogens and faecal pollution (Morgan et al., 2004).

*E. coli* is able to acquire resistance easily; therefore it is a good bio indicator model for surveillance studies of antimicrobial resistance. Antimicrobial resistance testing was performed by disc diffusion method using 7 different antibiotics for *E. coli*. Amoxicillin (7.4%), Ceftriaxone (3.7%) showed intermediate sensitivity but Ampicillin, Amoxicillin, Colistinsulphate, Ceftriaxone, and Gentamicin showed 81.48%, 70.3%, 74.02%, 70.3%, 7.42% resistance respectively. The resistance of Ceftriaxone, Ampicillin and Amoxicillin was supported by Niranjan and (Malini, 2013). The resistance against ampicillin was 81.48% in our study that was very close to the findings of (Boris et al., 2010) 85% and (Niranjan and Malini, 2013) 88.4%. Amoxicillin resistance was frequent and is around 70.30%, which is higher than the result of (Danishta et al., 2010) 21.5%. In our study isolates of *E. coli* showed 74.02% resistance against Colistinsulphate which was not corresponded with the result of (Kwa et al., 2005). Cephalosporin the most effective drug against different diseases of humans and animals are being used now-a-days. But in our study we found 70.3%Ceftriaxone resistant *E. coli* which was lower than the findings of (Odusanya et al., 2002) which was 77.6% and close to the findings of Niranjan and Malini (2013) which was 71.4%. In this study 7.42% resistance to gentamicin which was lower than 21.8% the result found by Seepersadsing et al. (2003). For Gentamicin, Enrofloxacin and Cephradine the organism showed 92.59%, 100%, 100% sensitivity respectively. A lower sensitivity
(58%) to Fluoroquinolones (Enrofloxacin) was observed than (Boris et al., 2010).

**Conclusion**

Jar Water is popular type of potable water source. Water intended for human consumption must be free of pathogenic and chemical agents, pleasant to taste and usable for domestic purposes. Since water is the most important potential source of infectious diseases so water purification is the most important potential available for ensuring public health. The present study showed that Jar water is likely to be contaminated by fecal Coliforms. Authorities need to minimize this contamination by maintaining cleaning-in-place (CIP) program, tight covering, routine disinfection, and fixing water taps underneath each jar to work as an outlet for drinking water. Public and Environmental Health and Regulatory Agency in Bangladesh needs to enforce effective monitoring of potable water and to spread culture of sanitation in the community.

**References**


