International Food Research Journal 25(2): 597-601 (April 2018)
Journal homepage: *http://www.ifrj.upm.edu.my*

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

[1*]Ahmad, M., [4]Islam, M. S., [2]Sarwar, N., [3]Kobra, K.T., [4]Hoque, M.M., [5]Junayed, M., [1]Mamun, A. A. [1]Morshed, S. and [3]Ahmed, T.

[1]*Department of Applied Chemistry and Chemical Technology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh*
[2]*Department of Food Processing and Engineering, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh*
[3]*Department of Applied Food Science and Nutrition, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh*
[4]*Department of Pathology and Parasitology, Chittagong Veterinary and Animal Sciences University, Khulshi, Chittagong-4225, Bangladesh*
[5]*Forest Protection Division, Bangladesh Forest Research Institute, Chittagong-4211, Bangladesh*

Article history

Received: 12 December 2016
Received in revised form: 2 January 2017
Accepted: 5 January 2017

Keywords

Jar water
MPN
PCR
Zoonotic pathogen

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-Khatibet *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

*Corresponding author.
Email: *ahmadmonsurfst@gmail.com*

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 (Armstronget *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 (Nwosuvc, 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 colioforms. 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 37^0C 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 37^0C 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 37^0C 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^{0}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 16 SrRNA 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^{0}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^{0}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. Gentamicin was resistant in 2 (7.42%) samples, whereas no resistance 0 (0%) was shown against Enrofloxacin and Cephradin.

Table 1. Isolation and identification of *Escherichia coli* and *Salmonella* spp.in culture on in MPN count, Nutrient agar, MacConkey agar, XLD agar, Indole test, TSI slant, PCR and stereotyping

Organisms	Nutrient agar	MacConkey agar	XLD agar	Indole test	TSI slant	PCR	Stereot-yping
E.coli	41 (21%)	41 (21%)	N/A	27 (14%)	N/A	27 (14%)	N/A
Salmonells Spp.	41 (21%)	41 (21%)	0.0	0.0	0.0	N/A	0.0

Table 2. Percentage of different patterns of Antimicrobial sensitivity test for *E. coli.*

Antibiotics	No. of samples (n=27)	Resistance (%)
AMP (Ampicillin)	22	81.41
AML (Amoxicillin)	19	70.30
CT (Colistinsulphate)	20	74.02
CN (Gentamicin)	2	7.42
ENR (Enrofloxacin)	0	0.0
CRO (Ceftriaxone)	0	0.0
CE (Cephradine)	19	70.30

Resistance to ≥1 antibiotic Multiresistance

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

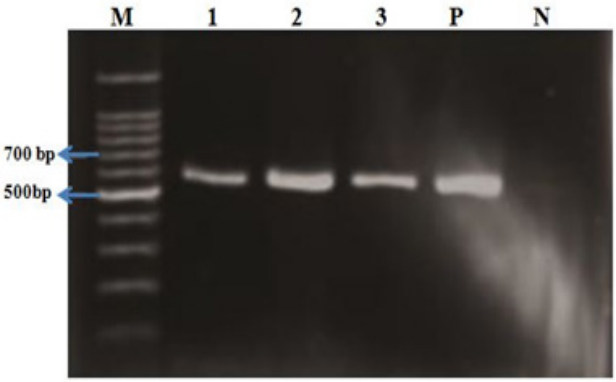

Figure 1. Results of PCR for 16s rRNA gene of *E. coli*

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 of the research done by (Nam *et al.*, 2010) and (Adesiyun *et al*., 1997) result 24.5%, but bit higher than 3.8% 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

Adesiyun, A. A., Webb, L. A., Romain, H. and Kaminjolo, J. S. 1997. Prevalence and characteristics of strains of *Escherichia coli* isolated from milk and feces of cows on dairy farms in Trinidad. Journal of Food Protection 60(10): 1174-1181.

Al-Khatib, I., Kamal, S., Taha, B., Al Hamad, J. and Jaber, H. 2003. Water-health relationships in developing countries: A case study in Tulkarem district in Palestine. International Journal of Environmental Health Research 13(2): 199-206.

Ahaduzzaman, M., Hassan, M. M., Alam, M., Islam, S. K. M. A. and Uddin, I. 2014. Antimicrobial resistance pattern against *Staphylococcus aureus* in environmental effluents, Research Journal for Veterinary Practitioners 2(1): 13-16.

Armstrong, L.E., Buyckx, M., Campbell, S. and Fulgoni, V. 2007. Scientific consensus statement regarding the importance of hydration and total water intake for health and disease. Journal of the American College of Nutrition 26 (5): 232-233.

Chao, K. K., Chao, C. C. and Chao, W. L. 2003. Suitability of the traditional microbial indicators and their enumerating methods in the assessment of fecal pollution of subtropical freshwater environments. Journal of Microbiology, Immunology and Infection 36(4): 288-293.

Cheesbrough, M. 2000. Microbiological tests. District laboratory practice in tropical countries. In Chaudhry, N. M. A. Tariq, P. (Eds). Anti-microbial activity of Cinnamomum cassia against diverse microbial flora with its nutritional and medicinal impacts, p. 43-53. New York: Cambridge University Press.

Daud, W. M. A. W., Ali, W. S. W. and Sulaiman, M. Z. 2001. Effect of carbonization temperature on the yield and porosity of char produced from palm shell. Journal of Chemical Technology and Biotechnology 76(12): 1281-1285.

Edberg, S.C., Alien, M.J., Smith, D.B. and Kriz, N.J. 1998. Enumeration of total coliforms and *Escherichia* colifrom source water by the defined substrate technology. Applied and Environmental Microbiology 56: 366-336.

Habrun, B., Kompes, G., Cvetnić, Ž., Špičić, S., Benić, M. and Mitak, M. 2010. Antimicrobial sensitivity of *Escherichia coli, Salmonella* spp., *Pasteurella multocida, Streptococcus suis* and *Actinobacillus pleuropneumoniae* isolated from diagnostic samples from large pig breeding farms in Croatia. Veterinarski arhiv 80(5): 571-583.

Johnson, J.M., Thomas, J.E., Graham, T.A., Townshend, I., Byrne, J., Selinger, L.B. and Gannon, V.P.J. 2003. Prevalence of *Escherichia coli* 0157:H7 *Salmonella* spp. in surface waters of southern Alberta and its relation to manure sources: Canadian Journal of Microbiology 49: 326-335.

Kwa, A.L., Loh, C., Low, J.G., Kurup, A. and Tam, V.H. 2005. Nebulized colistin in the treatment of pneumonia due to multidrug-resistant *Acinetobacterbaumanni*i and *Pseudomonas aeruginosa*. Clinical Infectious Diseases 41: 754-757.

Morgan, C.A., Bigeni, P., Herman, N., Gauci, M., White, P.A. and Vesey, G. 2004. Production of precise microbiology standards using flow cytometry and freeze drying. Cytometry 62A: 162–168.

Nam, H.M. 2010. Prevalence of antimicrobial resistance in fecal *Escherichia coli* isolates from stray pet dogs and hospitalized pet dogs in Korea. Microbial Drug Resistance 16: 75–79.

Niranjan, V. and Malini, A. 2014. Antimicrobial resistance pattern in *Escherichia coli* causing urinary tract infection among inpatients. The Indian Journal of Medical Research 139(6): 945-948.

Nwosuvc. 2001. Antibiotic resistance with particular reference to soil microorganisms. Research in Microbiology 152: 421-430.

Oblinger, J.L. and Koburger, J.A. 1975. Understanding and Teaching the Most Probable Number Technique. Journal of Milk and Food Technology 38(9): 540-545.

Ukhun, M.E., Tobi, S.B. and Okolie, N.P. 2005. Toxic chemicals and microbes in some Nigerian water samples. Journal of Medical Science 5: 260-265.

World Health Organization. 2003. The World Health Report 2003: shaping the future. World Health Organization (WHO).