American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS)
ISSN (Print) 2313-4410, ISSN (Online) 2313-4402

http://asrjetsjournal.org/

Bacteriological Evaluation of Drinking Water of Rajshahi City, Bangladesh

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Abstract

Access to pathogen free safe drinking water is one of the major challenges of the 21st century for most of the people of world. According to WHO guidelines, 100 ml sample of safe drinking must be free of total coliform, fecal coliform and *E. coli.* Hence, this study was designed for bacteriological evaluation of drinking water collected from different restaurant in Rajshahi city, Bangladesh. Among 10 samples, no detectable *E. coli* was found in 5 water samples (100 ml each) while 8-50 detectable *E. coli* in 100 ml water sample was found in the remaining 5 samples which exceeded the BDS and WHO standard of drinking water. Four randomly selected isolates (B_1, B_2, B_3 and R) which were isolated from different water samples were subjected to 16S rRNA gene sequencing. It was found that the 16S rRNA sequence of isolate B_1, B_2 and B_3 had 98% similarity to that of *Enterobacter asburiae*, *Escherichia coli* and *Acinetobacter sp* respectively. Similarly, 16S rRNA sequence of isolate R had 99% similarity to that of *Klebsiella pneumonia*. Results of biochemical analysis revealed that all four isolates were negative to Catalase, KOH and Oxidase test while variations were found for other tests. Similarly, all four isolates were able to ferment all tested carbohydrates except cellulose. The optimum pH and temperature for the growth of the all isolates was *pH* 7.0 and 37ºC. It was found that all the isolates were sensitive to majority of the antibiotics used.

Keywords: Bacteria; Pollution; drinking water; Rajshahi.

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1. Introduction

Water is essential for sustaining all life forms. An average human body contains 42 liters of water. With a loss of 2.7 liters, one can suffer from dehydration, weakness and headaches and consequently reach a state of pathology [1]. But, access to safe drinking water is one of the major challenges of the 21st century for most of the people of world. According to United Nations Millennium Declaration, 2000, access to clean and safe drinking water is a basic human need and microbiological control of drinking water should be the norm everywhere[2]. Reservoirs (dams), irrigation canals, wells and other withdrawal activities show that human have a manipulating and important impact on water cycle[3]. Displacements of people, loss of wild life and continuous alteration in river ecology and hydrology is the another effect[4]. The fecal contamination of the fresh water sources also poses a major threat to the mankind. Direct discharge of domestic wastes leaching from poorly maintained septic tanks and improper management of farm wastes are suspected as the major sources of water borne disease[5, 6]. Many developing regions suffer from the lack of safe drinking water for their population. About 800 billion people in Asia and Africa are living without access to safe drinking water consequently this has caused many people to suffer from various waterborne disease[7]. Mortality and morbidity from waterborne disease can be very high. The World Health Organization's (WHO) World Health Report for 1996 (APHA, 1995) estimates total mortality from diarrheal disease at over 3 million cases for 1995, with more than 80% among children under age 5. Food borne outbreaks of infectious disease can of course originate through food preparation with contaminated water. Low levels of pathogens in drinking water may rapidly multiply to infectious doses when associated with food. In addition, a susceptible host can become infected from drinking water and subsequently spread disease to others through person to person contact[8]. Provision of microbiologically safe drinking water therefore has dramatic impacts not only on incidence of waterborne disease but also on secondary transmission pathways. The most important bacterial gastrointestinal diseases transmitted through water are cholera, HUS, salmonellosis and shigellosis. The usefulness of indicator bacteria in predicting the presence of pathogens was well illustrated in many studies, namely by Wilkes and his colleagues 2009[9]. Routine basic microbiological analysis of drinking water should be carried out by assaying the presence of *Escherichia coli* by culture methods. According to the American legislation, total coliforms are the routine parameter to be determined. When these determinations are repeatedly positive, it is mandatory to assess fecal coliforms[10, 11]. Based on WHO and Indian drinking water standard, drinking water must be without any fecal coliforms bacteria in each 100 ml of water sample[12, 13]. However, the presence of pathogenic bacteria in water is sporadic and erratic. Hence, isolation and culture of these bacteria is not straight forward. For these reasons, routine microbiological analysis of water does not include the detection of pathogenic bacteria. But, safe water demands that water is free from pathogenic bacteria[14]. Lastly, pathogens in water derived from fecal contamination are the principal concerns in setting health-based targets for microbial safety[15, 16]. Therefore, regular bacteriological evaluation of drinking water of an area is a prerequisite for ensuring safety of human life of that area. But, recent information on bacteriological evaluation of drinking water in Rajshahi city, Bangladesh is unavailable while around 1 million residents of this city are drinking this water. Hence, this study was aimed to evaluate bacteriological contamination in drinking water available in different restaurants in Rajshahi city, Bangladesh.

2. Materials and methods

2.1 Sample collection

Total 10 samples were collected from different restaurant of Rajshahi university campus and central city of Rajshahi. Experiments were carried out within 1-4 hours after collecting the samples. As a negative control blank sample (autoclaved distilled water) was used in this investigation. All the samples were kept at 4ºC until these were analyzed.

2.2 Most Probable Number (MPN) technique

This technique was used to detect the fecal coliforms. The test was performed sequentially in three stages namely the presumptive, confirmed, completed tests. Dehydrated double and single strength MacConkey liquid media (Himedia) and EMB agar media (Himedia) were used in these experiments.

2.3 Presumptive test

Three set of tubes each containing three test tubes and Durham's tube was used in this experiment. First set containing 10.0 ml of double strength MacConkey liquid media were inoculated aseptically with 10.0 ml of water sample. Similarly, 2nd and 3rd sets containing 5 ml of single strength MacConkey liquid media were inoculated aseptically with 1.0 ml and 0.1 ml of water samples respectively. Then, tubes were incubated at 37°C for 2 days. Tubes were then observed for gas production after 24 and 48 hours. The presence of gas in any tube after 24 hr. was a positive presumptive test, the numbers of tubes in each set showing gas production were counted and the most probable count number/100 ml of the water sample was calculated by comparing with McCrady Chart, following the standard methods for examination of water given by APHA[17].

2.4 Confirmed test

This test was applied to all samples that give a positive or doubtful presumptive test. Inoculum from the MacConkey liquid media tube showing positive presumptive test with least quantity of water sample, was taken and streaked onto a plate of Eosin methylene blue (EMB) agar and kept for over-night incubation at 37°C. If typical dark colored colonies with green metallic sheen developed (most probably colonies of *E. coli*) on the plate within this period, the confirmed test was considered to be positive.

2.5 Completed test

From the EMB-agar plates, a single dark colored colony with metallic sheen (most probably colony of *E. coli*) was picked up and inoculated into 5ml peptone water and incubated at 37°C. After 4 hr. of incubation of peptone water at 37°C, inoculum from the incubated peptone water was inoculated on to citrate slope. Then**,** **i**noculated citrate media is incubated at 37°C, in an incubator, and the previously inoculated peptone water is further incubated at 44°C in a water bath for overnight incubation.

2.6 Biochemical tests

Since, bacteria *Escherichia coli* (*E. coli*) and *Enterobacter aerogenes* (*E. aerogenes*) bear a close resemblance to each other in their morphological and cultural characteristics, biochemical tests were performed to differentiate between them. The Triple Sugar Iron (TSI) Test, Simmons citrate test, Catalase Test, KOH Test, Oxidase Test were performed using protocol as previously described [18].

2.7 Fermentation test

Ability of the isolates for fermentation of Glucose, Fructose, Sucrose, Cellulose, Lactose, Maltose, Galactose and Xylose were tested using protocol as previously described[18].

2.8 Antibiotic sensitivity test

Sensitivity of antibiotic to the isolated bacteria was performed as described previously [19, 20]. Azithromycin Ciprofloxacin Ampicillin Cefradine Cefalexin Kanamycine Amoxyclav Doxycycline Streptomycin Gentamicin Erythromycin Tetracycline were used for test.

2.9 Effects of temperature and pH on bacterial growth

To determine the optimum pH of bacterial growth, culture medium was adjusted to pH 6, 7 and 8.0. For determination of optimum temperatures, inoculated media were incubated at 28°C, 37°C and 42°C. The growths of bacteria at different condition were determined at different time intervals by measuring optical density at 660 nm with photoelectric colorimeter.

2.10 Molecular characterization

Genomic DNA was extracted from dye decolourizing bacteria using CTAB method [21]. A universal PCR primer was used for amplification of 16S rDNA fragments. The protocol as previously described [22]. Briefly, the PCR amplification was performed by Swift™ Minipro Thermal Cycler (Model: SWT-MIP-0.2-2, Singapore) using the following program: Denaturing at 95°C for 5 minutes, followed by 40 cycles of 40 seconds of denaturing at 95°C, 60 seconds of annealing at 65°C and 2 minutes of elongation at 72°C with a final extension at 72°C for 10 minutes. Then, the PCR products were subjected to 1% agarose gel electrophoresis, stained with ethidium bromide and visualized on a UV transilluminator for the presence of about 1500 bp PCR products. PCR amplified 16s rDNA of the screened isolates was sent for automated sequencing (Applied Biosystem 3130) to the Centre for Advanced Research in Science (CARS) under Dhaka University, Bangladesh. The sequence generated from automated sequencing of PCR amplified DNA was analyzed through NCBI BLAST (http://www.ncbi.nlm.nih.gov) program to find out possible similar organism through alignment of homologous sequences. Finally, the isolates were identified based on alignment of partial sequence of 16S rDNA with the existing sequences available in the database.

3. Results

The presence of *E. coli*, an ideal indicator organism, in water sample indicate the water might be contaminated with pathogenic organism. According to WHO (1996) and BDS (1240:2001), the standard cfu of *E. coli* per 100ml is '0'. From the results of MPN it was found that among 10 samples 5 samples were within BDS and WHO standard while remaining 5 samples exceeded the standard (Table 1).

Table1: Load of *E. coli* (cfu) in drinking water in Rajshahi City.

Serial No.	**Site of Location**	***E. coli* (MPN/100ml)**	**BDS standard and WHO guide line 0 cfu /100ml**
1.	Rahmania restaurant	36	Value exceed BDS Standard and WHO guideline
2.	Biddut restaurant	50	Value exceed BDS Standard and WHO guideline
3.	Court Bazar restaurant	Nil	Values within BDS Standard and WHO guideline
4.	Rail Station footpath restaurant	17	Value exceed BDS Standard and WHO guideline
5.	Tripti restaurant	11	Value exceed BDS Standard and WHO guideline
6.	Memori biryani house restaurant	8	Value exceed BDS Standard and WHO guideline
7.	Madaripur restaurant	Nil	Values within BDS Standard and WHO guideline
8.	Monir restaurant	Nil	Values within BDS Standard and WHO guideline
9.	Sagor canteen	Nil	Values within BDS Standard and WHO guideline
10.	Bottola Tahari house restaurant	Nil	Values within BDS Standard and WHO guideline

Four isolates (B_1, B_2, B_3 and R) were subjected to 16S rRNA gene sequencing. The use of 16S rRNA gene sequences to identify bacteria has been certainly the most common method[23]. Hence, the isolated bacteria were subjected to 16S rRNA sequence based identification. Interestingly, the sequence analysis and subsequent BLASTn analysis indicated that the 16S rRNA sequence of isolate B_1, B_2 and B_3 collected from drinking water had 98% similarity to that of *Enterobacter asburiae*, *Escherichia coli* and *Acinetobacter sp* respectively (Table 2; Fig. 1, 2 and 3). Similarly, 16S rRNA sequence of isolate R had 99% similarity to that of *Klebsiella pneumonia* (Table 2; Fig. 4).

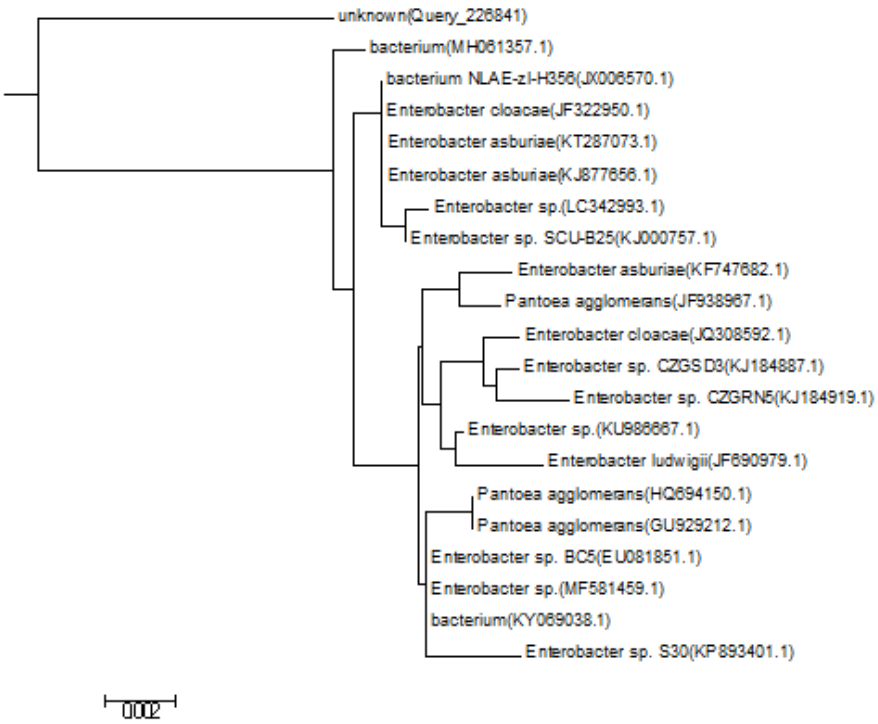

Figure 1: Unrooted Phylogenetic tree showing the genetic relationship between the isolate B_1 collected from drinking water and reference 16S rDNA sequences from the GenBank based on partial 16S ribosomal RNA gene sequences.

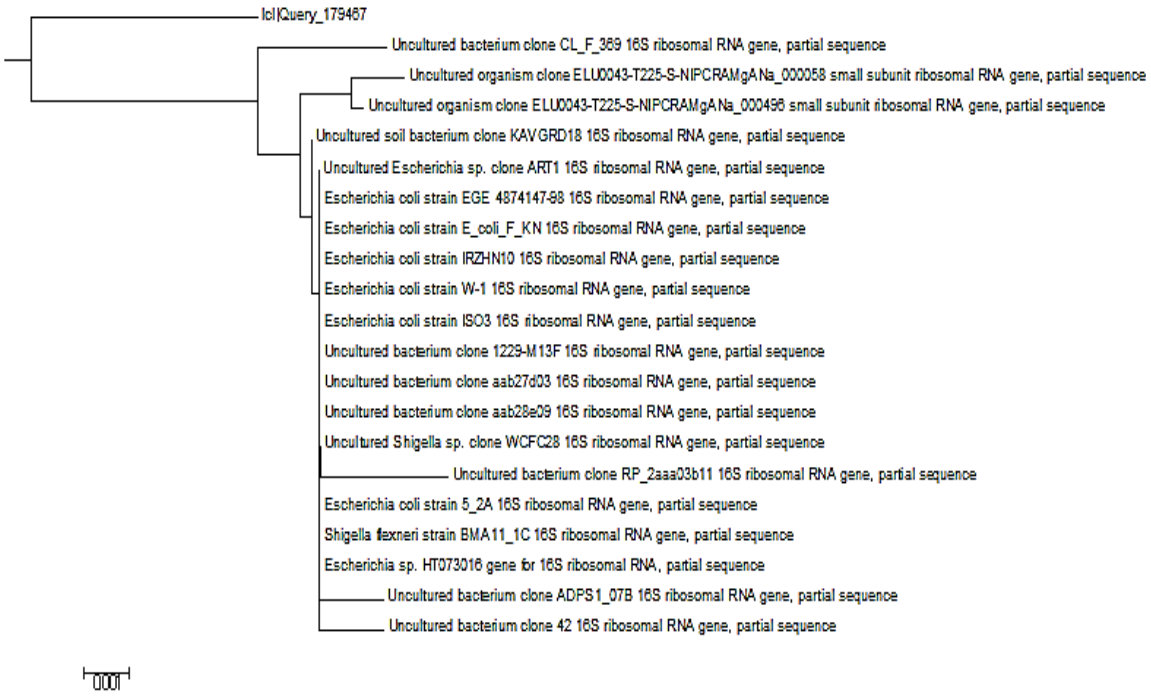

Figure 2: Unrooted Phylogenetic tree showing the genetic relationship between the isolate B_2 collected from drinking water and reference 16S rDNA sequences from the GenBank based on partial 16S ribosomal RNA gene sequences.

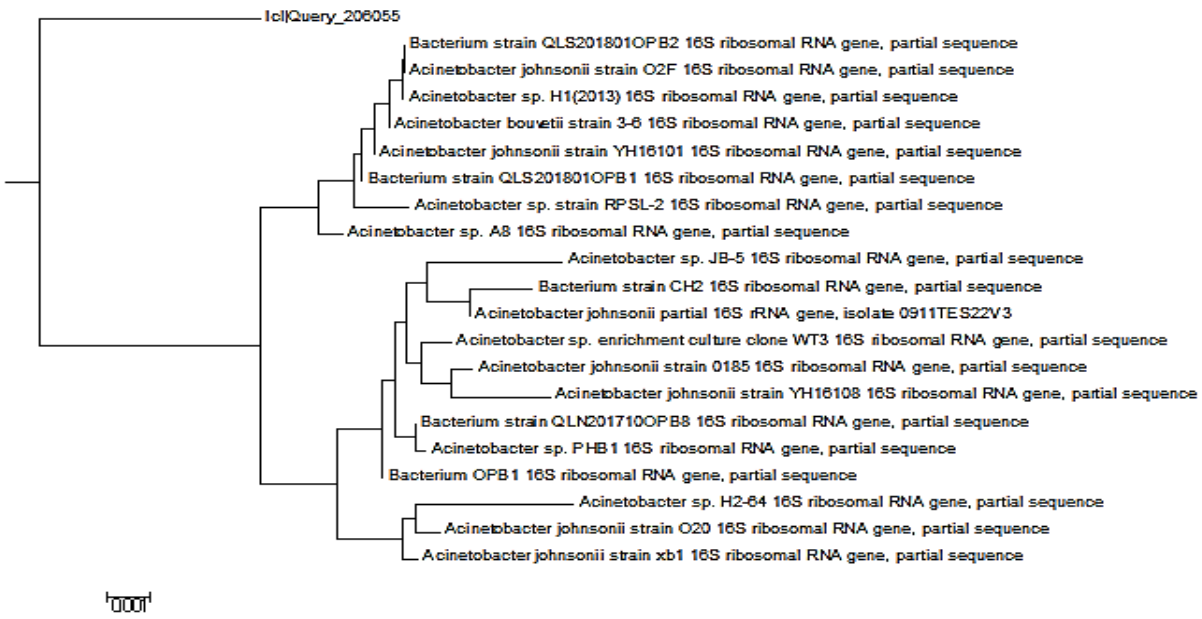

Figure 3: Unrooted Phylogenetic tree showing the genetic relationship between the isolate B_3 collected from drinking water and reference 16S rDNA sequences from the GenBank based on partial 16S ribosomal RNA gene sequences.

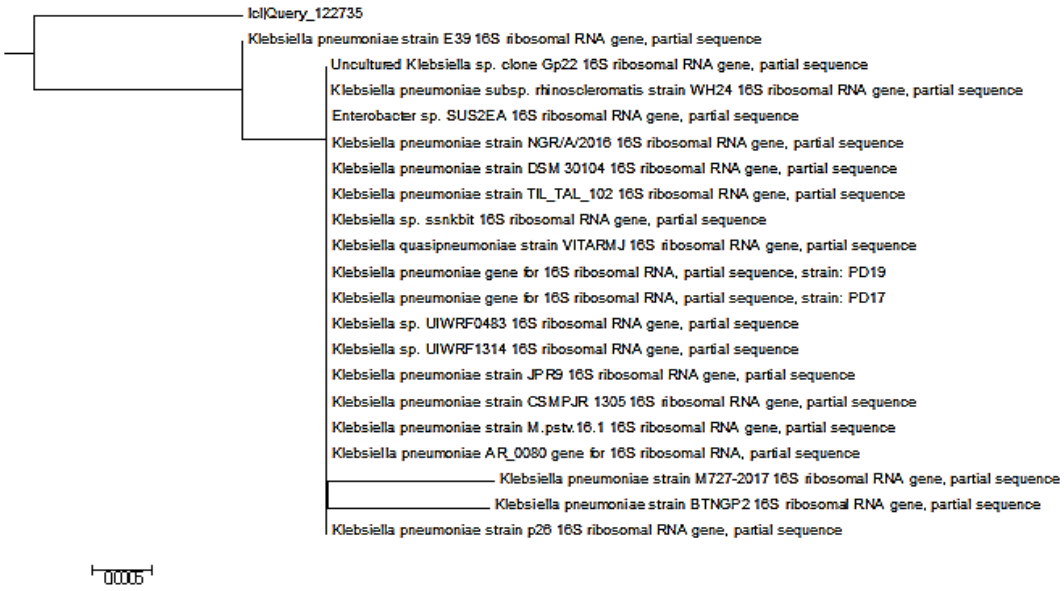

Figure 4: Unrooted Phylogenetic tree showing the genetic relationship between the isolate R collected from drinking water and reference 16S rDNA sequences from the GenBank based on partial 16S ribosomal RNA gene sequences.

Out of all isolates, one of the *E. coli* isolates (B_2) and three of other isolates (B_1, B_3 and R) were subjected to biochemical assays. Results of biochemical analysis revealed that all four isolates were negative to Catalase, KOH and Oxidase test while variations were found for other tests(Table 2). Similarly, all four isolates were able to ferment all tested carbohydrates except cellulose (Table 3).

Table 2: Result of biochemical studies.

Name of the tests	**B_1**	**B_2**	**B_3**	**R**
Triple sugar iron (TSI) test	+ve	+ve	-ve	+ve
Simmons citrate test	+ve	-ve	-ve	+ve
Sulfide indole motility (SIM) test	+ve	+ve	-ve	-ve
Catalase Test	-ve	-ve	-ve	-ve
Growth on MacConkey agar	+ve	+ve	-ve	+ve
KOH Test	-ve	-ve	-ve	-ve
Oxidase Test	-ve	-ve	-ve	-ve

Table 3: Results of fermentation test.

Sample ID	Fructose	Sucrose	Maltose	Cellulose	Galactose
B_1	+ve	+ve	+ve	-ve	+ve
B_2	+ve	+ve	+ve	-ve	+ve
B_3	+ve	+ve	+ve	-ve	+ve
R	+ve	+ve	+ve	-ve	+ve

The patterns of sensitivity and resistance of isolating bacterial cultures to 15 different antibiotics were tested by the disc method using on EMB (Eosin Methylene Blue) agar medium after overnight incubation at 37ºC. It was found that all the isolates were sensitive to majority of the antibiotics used (Table 4**).**

Table 4: Antibiotic sensitivity pattern of the isolates.

Antibiotics	**Zone diameter (mm)**			
	B_1	***B_2***	***B_3***	***R***
Azithromycin	15	15	5	15
Ciprofloxacin	25	28	25	39
Ampicillin	no zone	no zone	10	0
Cefradine	no zone	15	no zone	15
Cefalexin	no zone	no zone	no zone	no zone
Kanamycine	12	no zone	17	27
Amoxyclav	14	18	no zone	25
Doxycycline	15	20	21	22
Streptomycin	10	15	7	12
Gentamicin	no zone	15	no zone	15
Erythromycin	no zone	no zone	20	10
Tetracycline	18	25	24	25

(5-10 mm) = Resistance to antibiotic (R), (10-15 mm) = intermediate resistance (I) (15 -20 mm) = Sensitive to antibiotic (S), (>20 mm) = Hyper sensitive (H),

Effects of temperature and pH on bacterial growth:

The bacterial growth depended on the temperature and pH. The optimum pH for the growth of the isolates was 7.0. Bacterial growth was also recorded in other pH values ranging from 6.0 to 8.0 (Fig 5). The optimum temperature for the growth of bacteria was 37ºC and growth rate was moderately low in other temperatures such as 28ºC and 42ºC (Fig 6).

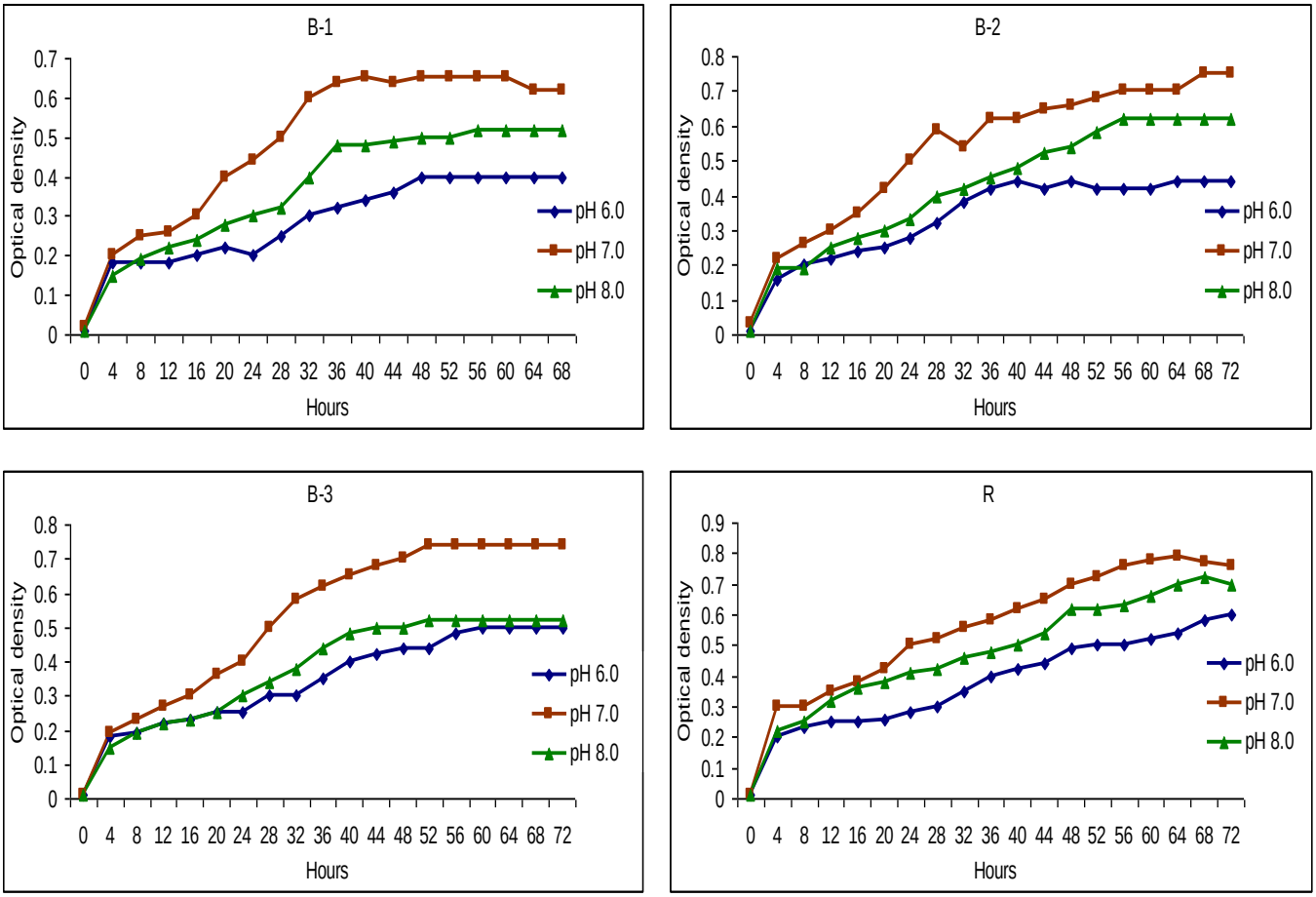

Figure 5: Effect of pH on bacterial growth of B_1,B_2,B_3 and R

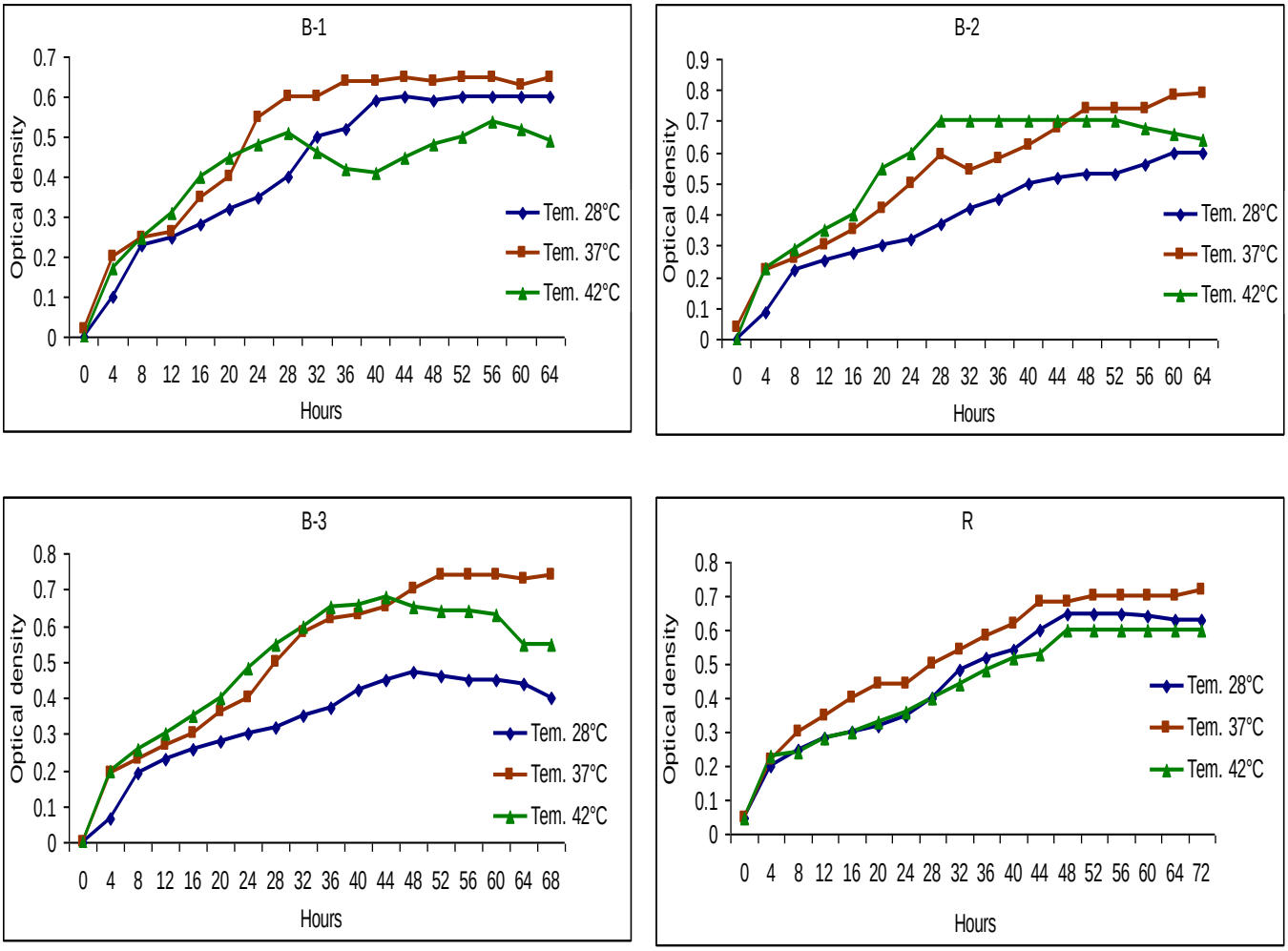

Figure 6: Effect of temperature on bacterial growth of B_1,B_2,B_3 and R

4. Discussion

The burden of waterborne diseases is a paramount in the globe. About 4% of the global burden diseases are attributable to water, sanitation and hygiene[24]. Nearly 2.2 million people die every year due to diarrhoeal diseases globally. Of these, 1.8 million deaths occur alone in low-income countries. Further, in low and middle-income countries one of the tenth leading causes of death is attributable to diarrhea- related disease[25]. It has been the goal of diagnostics to detect and enumerate target organisms in clinical and environmental samples rapidly and with sensitivity. Over the past ten years effective efforts have been made in this field[26]. Within the coliforms family *E coli* is of the prime interest and reliable indicator for fecal contamination rather than total coliform[27, 28], because its presence indicates recent fecal contamination with the possibility of enteric pathogens[29]. Assessment of water quality depends on detection of indicators in water samples[30]. In this study, it was found that 5 of 10 samples carried *E. coli* which is mostly concerned with fecal pollution[31, 32]. According to WHO, drinking water quality standards there should be no coliform as well as fecal coliform present in 100 ml or 0 colony forming units (cfu) per 100 ml (Health Services Academy 2005). Edberg and their colleague reported that *E. coli* survival depends upon environmental factors and type of water they mostly survived 4 to 12 weeks at moderate temperature[33]. In developed countries there are very rare cases to isolate

Salmonellae because of management of system[34]. Four isolates were considered for morphological, physiological and biochemical characteristics and they were gram negative, rod shaped and motile. pH values of drinking water in this study were within range which is mostly confirmed by other authors[35]. Bacteria survive greatly depend upon incubation temperature[36]. According to WHO, bacterial growth increases when temperature increases and it will lowers down when temperature drops [16]. Similar results were found in this study. All isolates grew luxuriantly at pH 7.0 and temperature 37°C which was comparable to other studies. Kirchman and Rich stated that bacterial species respond quickly to higher temperature when there is avalibilty of dissolved organic matter[37]. Bacteriological contamination in drinking water of different restaurant has remained one of the major problems in Rajshahi city. The chemical and biological contamination in drinking water is likely to occur due to leakage of pipes, pollution from sewerage, biofilm formation in the distribution system, intermittent water supply, and human activities[38, 39].

5. Recommendation

A regular monitoring for improvement of the water quality not only prevents disease and hazards but also checks the water resources from going further polluted. Moreover, the conservation of water sources is very important to provide safe water. Hence, development of regular monitoring system could be effective for protection of water sources from contamination by human and animal waste.

6. Conclusion

Around 50% of water sources of studied restaurants were contaminated with coliform bacteria indicating that bacteriological contamination in drinking water was one of the major problems in Rajshahi city, Bangladesh. It was found that the tested drinking water was contaminated with diverse types of bacteria such as *Enterobacter asburiae*, *Escherichia coli*, *Klebsiella pneumonia* and *Acinetobacter sp*. However, all the isolates were sensitive to majority of the tested antibiotics indicating their infection could be managed by antibiotics available in markets.

Reference

[1] J. B. Worfolk, "Heat waves: their impact on the health of elders," Geriatric Nursing, vol. 21, pp. 70-77, 2000.

[2] M. E. Assessment, Ecosystems and human well-being: wetlands and water: World Resources Institute, 2005.

[3] W. B. Solley, R. R. Pierce, and H. A. Perlman, Estimated use of water in the United States in 1995: US Geological Survey, 1998.

[4] D. Botkin and E. Keller, "Water supply use and management In," Environmental Science 5th ed. John Willey & sons, pp. 406-415, 2005.

[5] S. R. Huttly, "The impact of inadequate sanitary conditions on health in developing countries," World health statistics quarterly. Rapport trimestriel de statistiques sanitaires mondiales, vol. 43, pp. 118-126, 1990.

[6] I. George and P. Servais, "Sources et dynamique des coliformes dans le bassin de la Seine: Rapport de synthèse," 2002.

[7] F. Tanwir, A. Saboor, and M. Shan, "Water Contamination, health hazards and public awareness: a case of the urban Punjab, Pakistan," International Journal of Agriculture and Biology, vol. 5, pp. 560-562, 2003.

[8] W. H. Organization, "Molybdenum in drinking-water: background document for development of WHO guidelines for drinking-water quality," World Health Organization2003.

[9] G. Wilkes, T. Edge, V. Gannon, C. Jokinen, E. Lyautey, D. Medeiros, et al., "Seasonal relationships among indicator bacteria, pathogenic bacteria, Cryptosporidium oocysts, Giardia cysts, and hydrological indices for surface waters within an agricultural landscape," Water research, vol. 43, pp. 2209-2223, 2009.

[10] S. Pahuja, C. Tovey, S. Foster, and H. Garduno, "Deep wells and prudence: towards pragmatic action for addressing groundwater overexploitation in India," Deep wells and prudence: towards pragmatic action for addressing groundwater overexploitation in India., 2010.

[11] P. Wyrwoll, "India’s groundwater crisis," in Ground Water Forum, 2012.

[12] J. Bartram, J. Cotruvo, M. Exner, C. Fricker, and A. Glasmacher, Heterotrophic plate counts and drinking-water safety: IWA publishing, 2003.

[13] A. Amouei, M. B. Miranzadeh, Z. Shahandeh, T. Taheri, H. A. Asgharnia, S. Akbarpour, et al., "A study on the microbial quality of drinking water in rural areas of mazandaran province in north of Iran (2011)," Journal of Environmental Protection, vol. 3, p. 605, 2012.

[14] S. Saadi, M. Alimohammadi, R. Nabizadeh, A. Mesdaghinia, H. Aslani, S. Nazmara, et al., "Evaluating efficiency of radio waves for microbial removal in water samples," Journal of Advances in Environmental Health Research, vol. 2, pp. 157-164, 2014.

[15] P. Payment, M. Waite, and A. Dufour, "Introducing parameters for the assessment of drinking water quality," Assessing microbial safety of drinking water, vol. 4, pp. 47-77, 2003.

[16] M. Norisepehr, "Guidelines for Drinking Water: Hayyan publication," ed, 1994.

[17] S. Pathak and J. Bhattacherjee, "Effect of pollutants on survival of Escherichia coli in microcosms of river water," Bulletin of environmental contamination and toxicology, vol. 53, pp. 198-203, 1994.

[18] A. S. Chaity, Chowdhury, M.E.K., Hasan, M.F., Islam, M.A., Haque, M.F. and Sikdar, B. , "Isolation, characterization and control of canker disease causing bacteria of selected citrus plants in Rajshahi, Bangladesh," European J. Biotech. Biosc, , vol. 7, pp. 9-17, 2019.

[19] A. Saha, M. Haque, S. Karmaker, and M. Mohanta, "Antibacterial effects of some antiseptics and disinfectants," Journal of Life and Earth Science, pp. 19-21, 2009.

[20] M. K. Mohanta, A. K. Saha, M. F. Haque, S. A. Mahua, and M. A. Hasan, "Status of antibiotic sensitivity pattern of clinically isolated bacteria collected from Rajshahi City, Bangladesh," Univ. j. zool. Rajshahi. Univ, vol. 34, pp. 1-5, 2015.

[21] G. Smith, S. Socransky, and C. Smith, "Rapid method for the purification of DNA from subgingival microorganisms," Oral microbiology and immunology, vol. 4, pp. 47-51, 1989.

[22] A. K. Saha, N. Sultana, M. K. Mohanta, A. Mandal, and M. F. Haque, "Identification and characterization of azo dye decolourizing bacterial strains, Alcaligenes faecalis E5. Cd and A. faecalis Fal. 3 isolated from textile effluents," American Scientific Research Journal for Engineering, Technology, and Sciences (ASRJETS), vol. 31, pp. 163-175, 2017.

[23] J. M. Janda and S. L. Abbott, "16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls," Journal of clinical microbiology, vol. 45, pp. 2761-2764, 2007.

[24] A. Prüss, D. Kay, L. Fewtrell, and J. Bartram, "Estimating the burden of disease from water, sanitation, and hygiene at a global level," Environmental health perspectives, vol. 110, pp. 537-542, 2002.

[25] D. R. Murray and M. Schaller, "Historical prevalence of infectious diseases within 230 geopolitical regions: A tool for investigating origins of culture," Journal of Cross-Cultural Psychology, vol. 41, pp. 99-108, 2010.

[26] E. Humayun, A. Bibi, F. Akhtar, S. Ahmad, and N. Shujaat, "Isolation and identification of coliform bacteria from drinking water sources of Hazara Division, Pakistan," IOSR J Pharmacy, vol. 5, pp. 36-40, 2015.

[27] M. Stevens, N. Ashbolt, and D. Cunliffe, "Recommendations to change the use of coliforms as microbial indicators of drinking water quality," Australia Government National Health and Medical Research Council, 2003.

[28] D. Baudišová, "Evaluation of Escherichia coli as the main indicator of faecal pollution," Water Science and Technology, vol. 35, pp. 333-336, 1997.

[29] J. Jay, "Modren food microbiology 5th edition Van Nostrand Reinhold," New Yark, p. 661, 1996.

[30] J. Baudart, J. Coallier, P. Laurent, and M. Prévost, "Rapid and sensitive enumeration of viable diluted

cells of members of the family Enterobacteriaceae in freshwater and drinking water," Appl. Environ. Microbiol., vol. 68, pp. 5057-5063, 2002.

[31] A. Bej, M. Mahbubani, and R. Atlas, "Detection of viable Legionella pneumophila in water by polymerase chain reaction and gene probe methods," Appl. Environ. Microbiol., vol. 57, pp. 597-600, 1991.

[32] M. Petit, I. George, and P. Servais, "Removal of indicator bacteria, human enteric viruses, Glucoronidase activity measurements and characterization of cellular states'," Can. J. Microbiol, vol. 46, pp. 679-684, 2001.

[33] S. Edberg, M. Allen, and D. Smith, "Defined substrate technology method for rapid and specific simultaneous enumeration of total coliforms and Escherichia coli from water: collaborative study," Journal-Association of Official Analytical Chemists, vol. 74, pp. 526-529, 1991.

[34] C.-H. Chiu, C.-H. Chuang, S. Chiu, L.-H. Su, and T.-Y. Lin, "Salmonella enterica serotype Choleraesuis infections in pediatric patients," Pediatrics, vol. 117, pp. e1193-e1196, 2006.

[35] O. I. Omezuruike, A. O. Damilola, O. T. Adeola, E. A. Fajobi, and O. B. Shittu, "Microbiological and physicochemical analysis of different water samples used for domestic purposes in Abeokuta and Ojota, Lagos State, Nigeria," African Journal of Biotechnology, vol. 7, pp. 617-621, 2008.

[36] M. Sautour, P. Mary, N. Chihib, and J. Hornez, "The effects of temperature, water activity and pH on the growth of Aeromonas hydrophila and on its subsequent survival in microcosm water," Journal of Applied Microbiology, vol. 95, pp. 807-813, 2003.

[37] D. Kirchman and J. Rich, "Regulation of bacterial growth rates by dissolved organic carbon and temperature in the equatorial Pacific Ocean," Microbial Ecology, vol. 33, pp. 11-20, 1997.

[38] B. Chandio, "Groundwater pollution by nitrogenous fertilizers: A UNESCO Case Study," Water Resources Achievements and Issues in 20th Century and Challenges for Next Millennium, pp. 28-30, 1999.

[39] M. Tahir and M. Bhatti, "Survey of drinking water quality in the rural areas of Rawalpindi district. Pakistan Council of Research in Water Resources," Islamabad (Internal Report)1994.