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DIVERSITY AND ANTIBIOTIC SUSCEPTIBILITY OF BACTERIA IN WATER OF HOTEL RESTAURANTS IN DHAKA CITY

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Abstract

Present study was conducted to determine the microbiological status of water from dispensers in different roadside hotel and restaurants of Dhaka city. Samples were collected from seven hotel and restaurants. Aerobic heterotrophic bacterial count ranged between 1.5 × 10 and 8.8 × 10^3 cfu/ml. Enteric and related bacterial abundance in MacConkey, SS and Cetrimide agar media ranged from 0 to 4.9 × 10^6, 0 to 2.1 × 10^5 and 0 to 1.2 × 10^6cfu/ml, respectively. In total, 28 bacterial isolates were obtained during the study period. Among them, 15 were heterotrophic isolates and 13 were enteric and related bacteria. Among 15 aerobic heterotrophic isolates, 11 were gram positive and five were gram negative. Out of 11 gram positive isolates, 7 belonged to the genus *Bacillus viz. B. circulans, B. subtilis, B. stearothermophilus, B. brevis* and *B. cereus* and one to coccus *viz*. *Micrococcus roseus*. The other gram positive species were *Kurtia gibsonii*, *Auriobacterium liguefaciens* and *Curtobacterium luteum*. Four gram negative isolates were *Neisseria elongate* sub. spp. *glycolytica, Plesiomonas shigelloides, Pseudomonas fluorescens* biovar 1, *Pseudomonas aeruginosa.* All 13 enteric and related isolates were gram negative, short rod; and non-spore formers and belonged to the genera *Escherichia*, *Klebsiella, Shigella* and *Pseudomonas.* Among all isolates, two were resistant and six were susceptible to all five antibiotics.

Key words: Bacteria, Drinking water, Hotels, Restaurant, Dhaka city, Antibiotic resistant

Introduction

Transmission of pathogens through contaminated water is a significant cause of illness worldwide. It has been estimated that one-third of gastrointestinal illnesses are caused by contaminated drinking water (Hunter 1997), and 4% of all deaths worldwide are due to polluted drinking water and poor sanitation (Prüss *et al*. 2002). In developed nations water quality assessments and treatment facilities have been introduced to reduce microbial contamination, resulting in a significant reduction in drinking water-related illnesses and deaths. Water treatment commonly involves the reduction of organic matters and other contaminants via coagulation and sedimentation, separation of any remaining solids via filtration and finally disinfection using chemical oxidants or ultraviolet (UV) radiation. The addition of chemical oxidants such as chlorine and mono-chloramine is the most common method of drinking water disinfection (USEPA 1999).

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The level of treatment required varies from system to system, with some drinking water distribution systems receiving only one or two levels of treatment, while others require multiple treatments to create water suitable for end use.

Some members of the total coliform group are considerably more resistant to disinfection than *E. coli* and are better indicators of poor disinfection (WHO 2004 and Fricker and Eldred 2009). The presence of total coliforms in a water distribution system can also indicate a lack of system integrity (Besner *et al*. 2002). Thus, total coliform bacteria are commonly used to evaluate the general sanitary quality of water (WHO 2004 and Fricker and Eldred 2009).

Outbreaks of water borne diseases continue to occur throughout the world but especially serious in developing countries (WHO 1993 and Reynolds *et al*. 2007). Diarrheal diseases are endemic in Bangladesh. In 2008, an estimated 20,000 children less than 5 years old died of diarrheal diseases in Bangladesh (Huda *et al*. 2012). *E. coli* is commonly isolated from water sources, including the municipal water supply of Dhaka city (Islam *et al*. 2010). One study from India and another from Canada also reported the presence of antibiotic resistant *E. coli* in drinking water (Coleman *et al*. 2012 and Pathak and Gopal 2008). Armstrong *et al*. (1981) found that multiply antibiotic resistant (MAR), Gram positive cocci, e.g. *Staphylococcus* and MAR gram negative, non-fermentative rods *Pseudomonas*, *Alcaligenes*, *Moraxella*-like group M and *Acinetobacter* were more common in drinking waters than in untreated source waters.

According to the WHO guideline values for bacteriological quality, all water intended for drinking and treated water in the distribution system should not have detectable levels of *E. coli* or thermo-tolerant coliform bacteria in any 100 ml of the water sample (Sobsey and Bartram 2003). Unlike cholera, humans infected with salmonellae can carry the bacteria in the gut without signs of disease. Infected humans can harbor the bacteria for considerable periods of time. About 5% of patients clinically cured from typhoid fever remain carriers for months or even years. These people can be chronic holders of the bacterium in the gut, and constitute the main reservoir of the bacteria in the environment (Popoff and Le Minor 2005). Shigellosis or Bacillary dysentery is also an important waterborne disease which is an infectious disease caused by a group of bacteria called *Shigella*.

Dhaka is the 9thlargest city and also 28thamong the most densely populated cities in the world. Everyday lots of people take their meals from roadside hotel and restaurants. Besides high cost bottle water, low cost dispenser water is now very popular. The present study was undertaken for enumeration of both heterotrophic and enteric bacteriological abundance and comparison microbial abundances among those hotels and restaurants situated in Dhaka city.

Materials and Methods

Study site and sampling: In the present study water samples were collected from seven hotel restaurants in Dhaka city during the period from April 2013 to March 2014. Sterile plastic bottles were used for sample collection. Industrially prepared drinking water supplied to these restaurants were collected during sampling period.

Isolation of bacteria: Nutrient agar (NA) medium was used for the enumeration and isolation of aerobic heterotrophic bacteria, while MacConkey agar (Difco), SS agar (Diagnostic Pasteur), Cetrimide agar (Difco) media were used for the determination and isolation of enteric bacteria present in water samples. The pH of the medium was adjusted to 7, since pH of most of the samples were within the range of 6.58-6.95. Three different techniques *viz.* Pour plate technique (Greenberg *et al.* 1998), Spread plate technique (Sharp and Lyles 1969), Membrane filtration technique (Atlas *et al.* 1995) were used for the enumeration and isolation of bacteria. All the culture plates were marked with sample name and incubated at 37 °C in the dark for 48 hours. Bacterial colony counting was made with the help of a digital colony counter (DC-8 OSK 100086, Kayagaki, Japan). Discrete bacterial colonies were isolated immediately after counting. In case of MacConkey agar medium, pink or brick red colonies were considered as coliform bacteria while white colonies were considered as non-lactose fermenter, whereas in SS agar medium, black colonies were considered as highly pathogenic. In cetrimide agar medium, green colonies were considered *Pseudomonas* and pathogenic. During this investigation, of the total 40 isolates from nutrient agar medium, finally 28 isolates were randomly selected and purified for detailed study required for identification.

Physico-chemical properties of water: Temperature of Water samples was measured by a mercury centigrade thermometer.

(After collection of samples) pH was measured in the laboratory by an electric pH meter (Jenway 3310 pH meter, U.K).

Antibiotic sensitivity test: Five common antibiotics *viz.* Doxycycline. Penicillin G, Erythromycin, Gentamycin and Streptomycin were used to carry out the antibiotic sensitivity test.

Identification of bacteria: Important physiological and biochemical characteristics were studied for the identification of the selected isolates. Bergey's Manual of Systematic Bacteriology (Sneath *et al.* 1986) was followed for the identification of aerobic heterotrophic bacteria while, Manual for laboratory investigations of acute enteric infections (WHO 1987) and Bergey's manual of systematic bacteriology (Krieg and Holt 1984) were consulted for gram negative, enteric and related bacteria.

Results and Discussion

The physico-chemical properties of the samples are presented in Table 1. The water temperature ranged between 19ºC and 28ºC.Minimum water temperature was 19ºC recorded in sample No. 5. Maximum was 28ºC recorded in the sample No. 2 and sample No. 7. The pH of the sample water ranged between 6.58 and 6.95. Sample No. 7 showed the highest pH value (6.95) while the lowest pH value (6.58) was recorded in sample No. 6.

Table 1. Water temperature and pH of different samples.

Sample No.	Sampling sites	Water temperature (ºC)	pH
1	Star hotel and kabab	23	6.68
2	Rajdhani hotel and restaurant	28	6.60
3	Pita ghor hotel and restaurant	20	6.68
4	Al–Arju hotel and restaurant	25	6.90
5	Sayedabad hotel and restaurant	19	6.66
6	Hotel Kisukkhon	25	6.58
7	Allah'r dan hotel and restaurant	28	6.95

Table 2. Bacterial count (cfu/ml) of the water samples of different hotel restaurants.

Sample No.	Sampling sites	Aerobic heterotrophic bacteria	Enteric and related bacteria		
			MacConkey agar	SS agar	Cetrimide agar
1	Star hotel and Kabab	7.9×10^{2}	NG	NG	NG
2	Rajdhani hotel and restaurant	1.1×10^{3}	3.2×10^{2}	NG	NG
3	Pita ghor hotel and restaurant	8.8×10^{3}	1.8×10^{2}	2.0×10^{1}	NG
4	Al-Arju hotel and restaurant	1.3×10^{3}	3.0×10^{1}	1.8×10^{5}	1.2×10^{6}
5	Sayedabad hotel and restaurant	1.0×10^{3}	8.0×10^{1}	2.0×10^{1}	NG
6	Hotel Kisukkhon	1.5×10^{1}	2.4×10^{1}	1.7×10^{1}	4.1×10^{1}
7	Allah'r dan hotel and restaurant	1.1×10^{3}	4.9×10^{6}	2.1×10^{5}	NG

NG = No growth.

Aerobic heterotrophic bacterial count was higher than the count of enteric and related bacteria (Table 2). Aerobic heterotrophic bacterial count ranged between 1.5 × 10 and 8.8 × 10^{3} cfu/ml. In SS agar average bacterial count varied from 0 to 2.1 × 10^{5} cfu/ml. Bacterial count on MacConkey agar ranged between 0 and 4.9 × 10^{6}cfu/ml. In cetrimide agar medium bacterial count was within the range of 0 to 1.2 × 10^{6} cfu/ml and among the seven samples, 5 samples showed no bacterial growth (Table 2). Out of 15 selected

isolates, PG 11 and PG 23 were resistant, while RH 11, AD 11, SK 41, PG 21, RH 13 and HK 11 were susceptible to all five selected antibiotics (Table 3).

Table 3. Antibiotic sensitivity of the selected isolates.

Isolate No.	Inhibition zone measured in diameter (mm)				
	Name of the Antibiotics				
	E-15	P-10	GEN-10	S-10	DO-30
AD-10	R	R	S (18)	R	S (14)
PG-11	R	R	R	R	R
PG-22	S (20)	S (15)	S (29)	S (14)	S (20)
PG-23	R	R	R	R	R
RH-11	S (25)	S (12)	S (16)	S (12)	S (21)
AA-11	S (08)	R	S (22)	S (14)	S (10.5)
AA-13	R	R	S (22)	S (19)	S (11)
AD-11	S (24)	S (02)	S (19)	S (21)	S (15)
SK-41	S (13)	S (18)	S (13)	S (17)	S (17)
PG-21	S (21)	S (09)	S (17)	S (12)	S (19)
RH-13	S (12)	S (08)	S (14)	S (16)	S (14)
AA-12	S (03)	R	S (21)	S (21)	S (14)
SH-11	R	R	S (21)	S (14)	S (02)
SK-11	R	R	S (14)	R	S (18)
HK-11	S (14)	S (01)	S (14)	S (11)	S (15)

S = Sensitive, R = Resistant, E-15 = Erythromycin, P 10 = Penicillin G,
S-10=Streptomycin, GEN-10 = Gentamycin, N30 = Doxycycline.

Consulting all observed and tested characters of the bacterial isolates, identification was done. Ninety eight bacterial isolates were obtained during the study period. Among them 15 were heterotrophic isolates and 13 were enteric and related bacteria (Table 4). From the 15 aerobic heterotrophic bacteria 11 were gram positive bacterial isolates of which 7 belong to the genus *Bacillus* and one gram positive bacterial isolate to *Micrococcus.* Under the genus *Bacillus* the provisionally identified species were *Bacillus circulans* (two isolates), *Bacillus subtilis*, *Bacillus stearothermophilus*, *Bacillus brevis* and *Bacillus cereus* (two isolates). The four heterotrophic gram negative bacterial isolates were *Neisseria elongate* sub. spp. *glycolytica*, *Plesiomonas shigelloides*, *Pseudomonas fluorescens* biovar 1 and *Pseudomonas aeruginosa.* All 13 enteric and related isolates were gram negative, short rod and non-spore former and belonged to the genera *Escherichia coli*, *Klebsiella*, *Shigella* and *Pseudomonas.*

Table 4. Provisional identification of the selected bacterial isolates.

Isolate No.	Source (sample no.)	Species identified	
		Gram positive	Gram Negative
AD-10	7	*Aureobacterium liquefaciens*	–
PG-11	3	*Kurtia gibsonii*	–
PG-22	3	*Bacillus stearothermophilus*	–
PG-23	3	*Bacillus brevis*	–
RH-11	2	*Bacillus subtilis*	*Escherichia coli*
RH-17	2	–	*Klebsiella* sp.
AA-11	4	*Bacillus cereus*	–
AA-13	4	*Curtobacterium luteum*	–
AA-02	4	–	*Escherichia coli*
AA-18	4	–	*Shigella* sp.
AD-11	7	*Bacillus cereus*	–
AD-06	7	–	*Escherichia coli*
AD-14	7	–	*Shigella* sp.
SK-41	1	*Bacillus circulans*	–
PG-21	3	*Bacillus circulans*	–
PG-06	3	–	*Escherichia coli*
PG-02	3	–	*Shigella* sp.
RH-13	2	–	*Neisseria elongate* subspp *glycolytica*.
AA-12	4	–	*Plesiomonas shigelloides*
AA-03	4	–	*Pseudomonas aeruginosa*
SH-11	5	–	*Pseudomonas fluorescens* biovar 1
SH-21	5	–	*Escherichia coli*
SH-25	5	–	*Shigella* sp.
SK-15	1	–	*Pseudomonas aeruginosa*
HK-11	6	*Micrococcus roseus*	–
HK-05	6	–	*Pseudomonas aeruginosa*
HK-19	6	–	*Escherichia coli*
HK-22	6	–	*Shigella* sp.
AD-18	7	–	*Klebsiella* sp.

According to "WHO guidelines for drinking water quality", *Escherichia coli* (fecal coliform bacilli) must not be present in any 100 ml sample of (1) all water directly intended for drinking, (2) treated water entering the distribution system and (3) treated water in the distribution system. For effective disinfection, there should be a residual concentration of free chlorine of ≥ 0.5 mg / litre after at least 30 min contact time at pH <0.8. Chlorine residual should be maintained throughout the distribution system. At the point of delivery, the minimum residual concentration of free chlorine should be 0.2 mg/litre. The guideline value for chlorine used in water treatment that is of health significance in drinking-water is 5.0 mg / litre (WHO 2008). The results clearly showed that all the samples except sample no. 1 contaminated with *E coli*, *Shigella* sp., *Pseudomonas* and *Klebsiella* sp., which are pathogenic for human health. All the samples

showed presence of high number of bacteria as revealed by heterotrophic plate count, which is far beyond the limit set by WHO and USEPA for drinking water considered to be safe to public health.

References

Armstrong, J.L., D.S. Shigeno, J.J. Calomiris and R.J. Seidler. 1981. Antibiotic-resistant bacteria in drinking water.*Appl. Environ. Microbiol.* **42**(2): 277-283.

Atlas, R.M., A.E. Brown and L.C. Parks. 1995. *Laboratory Manual of ExperimentalMicrobiology*. Mosby-Year Book, Inc., St. Louis. pp. 1-565.

Besner, M-C., V. Gauthier, P. Servais and A. Camper. 2002. Explaining theoccurrence of coliforms in distribution systems. *J. AWWA* **94**:95–109.

Coleman, B.L., M.I. Salvadori, A.J. McGeer, K.A. Sibley and N.F. Neumann. 2012. The role of drinking water in the transmission of antimicrobial resistant *E. coli*.*Epidemiology and Infection* **140**: 633-642.

Fricker, C.R. and B.J. Eldred. 2009. Identification of coliform genera recoveredfrom water using different technologies. *Lett. Appl. Microbiol.* **49**: 685–688.

Greenberg, A.E., J.J. Connors, D.G.J. Jenkins and M.A.H. Franson. 1998. *Standard methods for examination of water and wastewater* (20th Ed.). APHA. Washington DC. p. 265.

Huda, T.M., L. Unicomb, R.B. Johnston, A.K. Halder, M.A.Y. Sharker. 2012. Interim evaluation of a large scale sanitation, hygiene and water improvement programme on childhood diarrhea and respiratory disease in rural Bangladesh. *Social Science & Medicine* **75**: 604–611.

Hunter, P.R. 1997. *Waterborne disease: epidemiology and ecology*. John Wiley and Sons, Chichester, United Kingdom.

Islam, S., H.A. Begum and N.Y. Mili. 2010. Bacteriological Safety Assessment of Municipal Tap Water and Quality of Bottle Water in Dhaka City: Health Hazard Analysis. Bangladesh.*J. Med. Microbiol.* **4**(1): 9-13.

Krieg, N.R. and J.G. Holt (Eds.). 1984.*Bergey's Manual of Systematic Bacteriology*. The Williams and Wilkins Company, Baltimore, USA. Vol.1: pp. 140-575.

Pathak, S.P., K. Gopal. 2008. Prevalence of bacterial contamination with antibiotic resistant and enterotoxigenic fecal coliform treated drinking water. *J. Toxicol. Environ. Health* **71**: 427-433.

Popoff, M.Y. and L.E. Le Minor. 2005. Genus *Salmonella*. *In*: *Bergey's Manual of Systematic Bacteriology* (2nd Ed), Vol. 2, Part B. Brenner, D.J., N.R. Krieg, J.T. Staley, G.M. Garrity, D.R. Boone, P.D. Vos, M. Goodfellow, F.A. Rainey and K.H. Schleifer (Eds). Springer.NY. pp. 764–799.

Prüss, A., D. Kay, L. Fewtrell and J.Bartram. 2002. Estimating the burden ofdisease due to water, sanitation and hygiene at a global level. *Environ Health Perspect*. **110**: 537–542.

Reynolds, K.A., K.D. Mena and C.P. Gerba. 2007.Risk of water-borne illness via drinking water in the United States.*Rev. Environ.Contam.Toxicol.* **192**: 117-158.

Sharp, M.S. and S.T. Lyles. 1969.*Laboratory Instruction in Biology of Microorganisms*.Saint Louis the CV Mosley Company. pp. 23-25.

Sneath, P.H.A., N.S. Mair, M.E. Sharpe and J.G. Holt (Eds.). 1986. *Bergey's manual of systematic bacteriology* (9thEd.). The Williams and Wilkins Co., Baltimore, USA. Vol. 2, p. 1599.

Sobsey, M.D. and S. Bartram. 2003. Water quality and health in the new millennium: the role of the World Health Organization Guidelines for Drinking-Water Quality. *Forum Nutr.* **56**: 396-440.

USEPA. 1999. *EPA guidance manual*: alternative disinfectants and oxidants, EPA 815-R-99-014. US Environmental Protection Agency, Washington, DC.

WHO. 1987. *Manual for laboratory investigations of acute enteric infections.*World Health Organization p. 1-109.

WHO. 1993. *Guidelines for drinking water quality* (Vol. 1). World Health Organization, Geneva. pp 1-29.

WHO. 2004. Guidelines for drinking-water quality, (3rd ed.), Vol 1. Recommendations. *World Health Organization*, Geneva, Switzerland.

WHO. 2008. *Guidelines for drinking-water quality*, (3rd Ed.). (Incorporating first & second addenda), Vol. 1 - Recommendations, Geneva.

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