The Bangladesh Veterinarian (2023) 40(1 – 2): 16 – 24

Detection of tetracycline and ampicillin resistant *E. coli* and *Salmonella* species from hospital wastewater

Mahmood S, Islam M, Chowdhury S[1] and Mannan MA*

Department of Microbiology and Parasitology, Faculty of Animal Science and Veterinary Medicine, Sher-e-Bangla Agricultural University, Sher-e-Bangla Nagar, Dhaka-1207, Bangladesh

Abstract

Escherichia coli (E. coli) and *Salmonella* spp. were found in hospital wastewater. The highest (E = (6.2 ± 1.8) x 10^9; S = (4.3 ± 2.2) x 10^9) and lowest (E = (1.4 ± 1.2) x 10^9; S = (1.1 ± 1.8) x 10^9) Total Viable Count (TVC) were in Shaheed Suhrawardy Medical College Hospital (SSMCH) and National Institute of Ophthalmology Hospital (NIOH), respectively.

The overall occurrence of *E. coli* and *Salmonella* spp. was 80% and 87%, respectively, with 100% occurrence in SSMCH and National Institute of Cardiovascular Diseases Hospital (NICVDH) for *Salmonella* spp. ($p \leq 0.036$). Prevalence of *E. coli* was low (72%) in winter (November - February). Both types of bacteria were resistant to Tetracycline whereas and 97.5% of E coli were resistant to ampicillin. Gentamycin was more effective than other antibiotics. Rational use of antibiotics is suggested, and hospital management practice should be followed as defined by the Joint Commission International for Hospital Accreditation. (*Bang. vet.* 2023. Vol. 40, No. 1 – 2, 16 – 24)

Introduction

Wastewater can be contaminated by natural disaster or manmade (Afroz *et al.,* 2014). Liquid waste discharged from domestic homes, agricultural or commercial, pharmaceutical sectors, hospitals, floods or earthquakes may be contaminants. Huge quantities of water are used in hospitals and are a major source of pathogens (Mahmud *et al.,* 2019). A lot of infectious agents spread through contaminated hospital waste water, which are responsible for waterborne diseases. Many non-metabolized drugs excreted from patients, and residual chemicals, enter wastewater, and interact with the micro-flora (Majumder *et al*., 2011 and Uddin, 2018).

Wastewater can cause nosocomial infection (Akther *et al.,* 2018). Water is mainly contaminated by *E. coli*, *Salmonella* spp., *Shigella* spp., *Streptococcus* spp., *Campylobacter* spp., *Bacillus* spp., *Pseudomonas* spp. and fungi (Munshi *et al*., 2012).

---

[1]Department of Pathology and Parasitology, Faculty of Veterinary Medicine, Chattagram Veterinary and Animal Sciences University, Zakir Hossain Road, Chattagram-4225, Bangladesh

*Corresponding author:- E-mail: 1971bangla@gmail.com; mam1982@sau.edu.bd;

Mobile: +8801818837088, +8801715264493

DOI: https://doi.org/10.3329/bvet.v40i1-2.71120

Received: 8 November 2023; **Accepted:** 9 December 2023; **Published:** 23 January 2024

Antibiotic resistant has become an alarming issue all over the world, and should be used only as prescribed. Wastewaters are considered important sources of bacterial resistance (Tesfaye *et al.*, 2019). Antibiotic resistant bacteria may spread to the patients, assistants, visitors, doctors or pet animals (Hocquet *et al.*, 2016).

Sewerage network of Sher-e-Bangla Nagar of Dhaka City, where there are many hospitals, has a drainage system connected with Turag and Buriganga Rivers. When untreated hospitals' wastewater enters the sewerage network, it mixes with the domestic sewage water. Many people living around these rivers use water for various purposes and get infection. Wastewater often contains multi drug resistant (MDR) *E. coli* and *Salmonella* spp. that can disseminate antibiotic resistance to other bacteria (Siddiqui *et al.,* 2015). Arthropods may transmit these bacteria from drains to open water, and transmit infection (Rahman *et al.*, 2017). There is no comprehensive precise data available for such infections. A study was undertaken to identify the *E. coli* and *Salmonella* spp. from wastewater samples of selected hospitals, with assessment of antibiotic resistance pattern of the isolates.

Materials and Methods

Study area and period

A cross-sectional study was designed to investigate the bacteria in hospital wastewater. Five hospitals were selected in Dhaka City. Samples were collected from SSMCH**,** National Institute of Cardiovascular Diseases (NICVDH)**,** National Institute of Traumatology and Orthopedics Rehabilitation (NITORH), NIOH and Bangladesh Shishu Hospital and Institute (BSHI) from November 2020 to June 2021. All hospitals and institutes are close to Sher-e-Bangla Nagar in Dhaka Metropolitan areas.

Study size and population

Twenty 200 ml wastewater samples were collected from each hospital. The samples were transferred into sterile test tubes and transported to the Microbiology and Parasitology laboratory of the Sher-e-Bangla Agricultural University for examination.

Culture in liquid and solid media

The liquid media were Nutrient broth (NB), Methyl-red (MR) and Voges-Proskauer broth (VP). The solid media were Nutrient agar (NA), MacConkey agar (MC), Salmonella-Shigella agar (SS) and Eosin Methylene Blue agar (EMB). The 100 microliters of the processed sample were inoculated into Nutrient broth followed by other solid media using spread plate technique as described by Hassan *et al.* (2015).

The inoculated media were incubated at $37^{o}C$ for 24 hours and observed every six hours. Cultural properties of the *Salmonella* and *E. coli* on liquid and various solid media were observed with the naked eye and microscopically.

Total Viable Count (TVC) and TCC counts

The TVC and TCC were counted for determination of bacterial load as described by Biyani *et al.* (2018).

Staining properties and microscopic observation

Gram's stain was done according to Hassan *et al.* (2015). The colonial morphology was recorded using microscope (100 x).

Biochemical properties

Catalase, Indole, Methyl Red (MR), and Voges-Proskauer (VP) tests were conducted as described by Cowan and Steel (1985) and Hassan *et al.* (2015).

Cultural sensitivity test

The sensitivity tests were done against Ampicillin, Ciprofloxacin, Gentamycin, Streptomycin and Tetracycline by disc diffusion method. The sensitivity patterns were analysed according to Clinical and Laboratory Standards Institute (CLSI, 2007) and zone diameter interpretive standards (Wilson *et al.*, 2007).

Table 1: The zone diameter interpretive standard for *E. coli* and *Salmonella* spp.

Antibiotic disc	Disc content	Resistant	Intermediate	Sensitive
Ampicillin	10 µg	≤ 12	13-19	≥ 20
Ciprofloxacin	5 µg	≤ 20	21-30	≥ 31
Gentamycin	10 µg	≤ 12	13-14	≥ 15
Streptomycin	10 µg	≤ 15	16-21	≥ 22
Tetracycline	30 µg	≤ 11	12-14	≥ 15

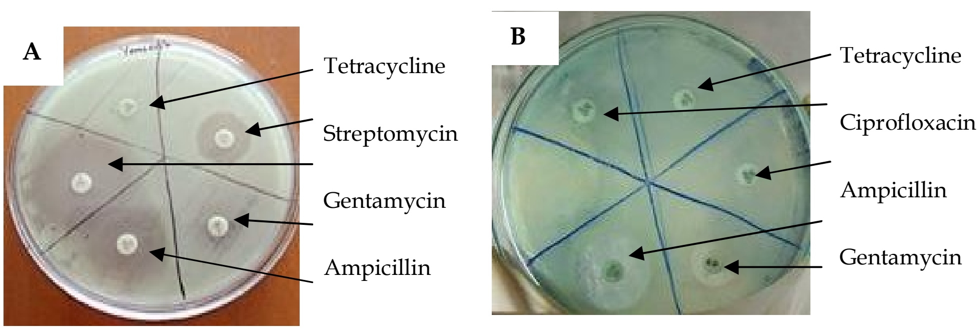

Fig. 1: Cultural sensitivity test for *E coli* (A) and *Salmonella* (B) in nutrient agar.

Data analysis: The data were analysed using SPSS software (version 20.0). The means and standard deviations were determined. The correlation of the bacterial load among the hospitals and seasons (winter: November to February; summer: March to June) was measured. P-value ≤0.05 was considered statistically significant.

Result and Discussion

Observation of the growth properties in various media

E. coli and *Salmonella* spp. were cultivated in the nutrient broth that produced turbidity in positive cases.

Culture of E. coli in solid agar media

The *E. coli* bacteria were inoculated in Nutrient agar, MacConkey agar and Eosin Methylene Blue (EMB) agar. The colonies were smooth, circular and white on Nutrient agar. The green metallic colonies were noted in EMB agar. In MacConkey agar, bright pink to red colonies were produced due to fermentation of lactose by *E. coli* (Fig. 2). EMB agar was used to differentiate the coliform enteric bacteria from other enteric bacteria as evidenced by the production of acid. In acidic conditions, the dyes produce a dark purple complex with a green metallic sheen.

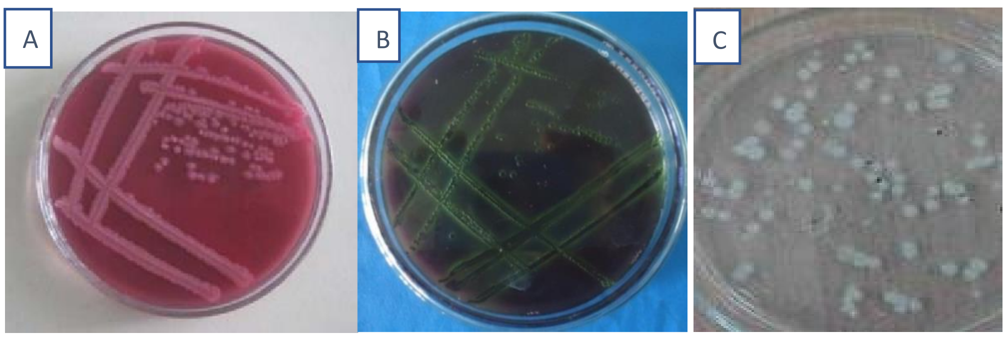

Fig. 2: Colonies of *E. coli* in MacConkey agar (A), EMB agar (B) and Nutrient agar (C).

Culture of Salmonella spp. in solid agar media

The colonies of *Salmonella* spp. in Salmonella-Shigella (SS) agar were opaque, translucent, colourless, smooth and round with black centres, as they were lactose non-fermented (Fig. 3). On EMB Agar, the *Salmonella* colonies were pinkish white or red with a red halo. Differentiation was quite pronounced as lactose or sucrose fermenting organisms, which produced yellow green colonies (Fig. 3).

Total Viable Count (TVC)

Total viable count differed between hospitals. The highest TVC was in SSMCH (6.2 ± 4.3) x10^9 cfu/ml and the lowest in NIOH (1.4 ± 1.2) x10^9 cfu/ml for *E. coli* (Table 2). The SSMCH is a teaching hospital and many patients come for general and special health care. NIOH is a specialized eye hospital with fewer patients. This may be the cause of higher count of TVC in teaching hospital than the specialized hospital. The observation of Sharmin *et al.* (2021) on TVC in Dhaka city was 7.04 ± 0.48 cfu/gm from water of street vendor. The result of this study showed lower numbers than earlier reports. Khalil and Gomaa (2014) reported wide range of TVC (log 3.63 – 7.17

cfu/gm). The TVC in different hospitals varied due to biosecurity, hygienic management, sources of water, drainage facilities, services pattern, and procedure followed during collection.

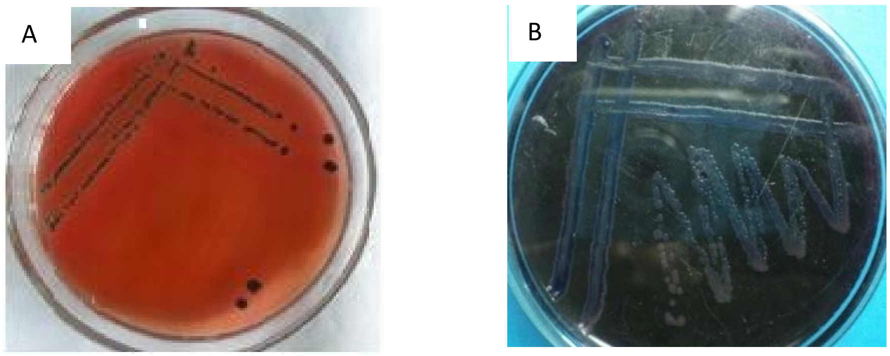

Fig. 3: Colonies characteristics of *Salmonella* spp. in SS agar (A) and EMB agar (B).

Table 2: Total Viable Count (TVC) from samples

Hospital	Number of samples	Total Viable Count (mean ± SD) in cfu/ml	
		E. coli	*Salmonella* spp.
SSMCH	20	$(6.2 \pm 1.8) \times 10^9$	$(4.3 \pm 2.2) \times 10^9$
NICVDH	20	$(2.8 \pm 2.1) \times 10^9$	$(3.2 \pm 2.3) \times 10^9$
NITOR	20	$(2.7 \pm 1.9) \times 10^9$	$(2.2 \pm 1.5) \times 10^9$
NIOH	20	$(1.4 \pm 1.2) \times 10^9$	$(1.1 \pm 1.8) \times 10^9$
BSHI	20	$(1.9 \pm 2.4) \times 10^9$	$(1.2 \pm 1.7) \times 10^9$

Staining properties and microscopic observation

The bacteria were Gram-negative, small, rod-shaped, pink, and arranged in pairs or short chains (Fig. 4).

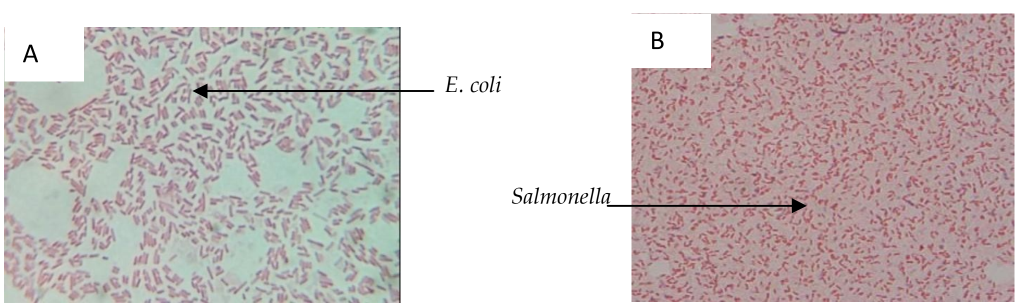

Fig. 4: Gram-negative, pink rod-shaped *E. coli* (A) and single or paired *Salmonella* (B) under the light microscope (100 x).

Biochemical observations

The *Salmonella* and *E. coli* bacteria showed red colour in MR positive test and colour was not changed in VP negative test. In catalase test, bubble formation was recorded as positive in both *Salmonella* and *E coli*. Red ring was formed in positive reaction in Indole test for *E. coli* and orange colour developed in *Salmonella* bacteria for negative cases (Table 3).

The cultural, staining and biochemical properties of *E. coli* and *Salmonella* were similar to the description of Ronald Atlas (2015).

Table 3: Biochemical properties of *E. coli* and *Salmonella* spp.

Sl. No.	Biochemical tests	Results	
		E. coli	*Salmonella* spp.
1	MR	+	+
2	VP	-	-
3	Indole	+	-
4	Catalase	+	+
5	Gas production	+	-

Epidemiological observation

The occurrence of *E. coli* and *Salmonella* spp. at different hospitals varied. The highest occurrence was at NICVDH (Table 4). The lowest were at NIOH. SSMCH had significantly higher occurrence of *Salmonella* species. Nahar *et al*. (2019) reported 76.6 and 89.3% *E. coli* and *Salmonella* spp., respectively, in environmental samples. Kam *et al.* (2007) isolated 134 (56%) *Salmonella* spp. from 17 hospitals and clinics in Hong Kong. This study was similar to their findings.

Table 4: Occurrence of bacteria from wastewater in different hospitals

Sl. No.	Hospitals	Total samples	*E. coli*			*Salmonella* spp.		
			Positive	Occurrence (%)	P-value	Positive	Occurrence (%)	P-value
1.	SSMCH	20	17	85	0.087	20	**100**	0.036
2.	NICVDH	20	18	**90**		20	100	
3.	NITORH	20	18	90		16	80	
4.	NIOH	20	12	60		15	75	
5.	BSHI	20	15	75		16	80	
	Total	100	80	80		87	87	

The highest occurrence was in summer for both types of bacteria. Winter was correlated with outbreak of *E. coli*. Nahar *et al.* (2019) recorded the occurrence of the *E. coli* (85%) and *Salmonella* (95%) spp. in sewage water in summer. The maximum load of *E. coli* and *Salmonella* spp. was similar to Nahar *et al.* (2019). The hospital waste and sewage water were always contaminated and might be considered as one major sources of contaminants.

Table 5: Seasonal occurrence of bacteria from wastewater in Dhaka city

Sl. No.	Seasons	Total samples	*E. coli*			*Salmonella* spp.		
			Positive	%	P-value	Positive	%	P-value
1.	Winter	50	36	72	0.046	42	84	0.37
2.	Summer	50	44	88		45	90	
	Total	100	80	80		87	87	

Cultural sensitivity tests

Sensitivity to Ciprofloxacin, Streptomycin, Ampicillin, Tetracycline and Gentamycin are shown in Table 6. There was no sensitivity (0.00%) to Ampicillin and Tetracycline for *E. coli* and *Salmonella* spp. Gentamycin showed 93.8% and about 80% for *E. coli* and *Salmonella* spp., respectively. All antibiotic discs showed a wide range (00% to 47%) of intermediate values against both types of bacteria. All Salmonella were resistant to Tetracycline and ampicillin. *E. coli* was 100% resistant to tetracycline and 97.5% to ampicillin.

Table 6: Antimicrobial profile of *E. coli* and *Salmonella* spp.

Name of antibiotic	Isolated bacteria	Sensitive	Intermediate	Resistant
Ciprofloxacin	E (80)	52 (65.0%)	28 (35.0%)	0 (0.00%)
	S (87)	65 (73.9%)	17 (19.3%)	6 (6.9%)
Streptomycin	E (80)	50 (62.50%)	18 (22.5%)	12 (15.0%)
	S (87)	30 (34.0%)	42 (47.8%)	16 (18.2%)
Ampicillin	E (80)	0 (0.00%)	2 (2.5%)	**76 (97.5%)**
	S (87)	0 (0.00%)	0 (0.0%)	**87 (100%)**
Tetracycline	E (80)	0 (0.00%)	0 (0.0%)	**80 (100%)**
	S (87)	0 (0.00%)	0 (0.0%)	**87 (100%)**
Gentamycin	E (80)	**75 (93.8%)**	5 (6.3%)	0 (0.00%)
	S (87)	**70 (79.5%)**	18 (20.5%)	0 (0.00%)

Legend: E = *E. coli*, S = *Salmonella* spp.

E. coli and *Salmonella* spp. was completely resistant to tetracycline, but *E. coli* was 97% resistant to ampicillin (Nahar *et al*., 2019). The multi-drug resistant (MDR) *E. coli* was reported by Rashid *et al.* (2015). Siddiqui *et al*. (2015) showed antibiotic-resistant *Salmonella* spp. in hospital waste and sewage.

Tetracycline is widely used by the veterinary and human practitioners in Bangladesh (Hassan *et al*., 2015). Many patients and farmers buy antibiotics from the local pharmacies without prescription. Continuous, long time and indiscriminate use of antibiotics might be the causes of MDR. Moreover, effluent treatment plants are not sufficient to cope with the hospital wastes.

Conclusions

The *E. coli* and *Salmonella* spp. were detected in SSMCH and NIOH in 80% and 87% of samples, respectively. Both were resistant to Tetracycline and ampicillin. Further study is needed with more samples covering hospitals in urban and semi-urban areas of Bangladesh.

Acknowledgments

The authors express cordial thanks to Rakib Hasan and Basant Saud from Microbiology and Parasitology department for continuous support. The funding from the Bangladesh University Grants Commission and Sher-e-Bangla Agricultural University Research System in 2020-21 was cordially acknowledged. The authors also express their heartiest thanks to Maheshwor Mali and Abul Kalam for technical support.

Conflicts of Interest

The authors declare that they have no conflict of interest.

Declaration

The authors declare that the manuscript is original and not previously published or under consideration for publication in any types of journal.

References

Afroz H, Hossain MM, Fakruddin M 2014: A 6-year retrospective study of bloodstream *Salmonella* infection and antibiotic susceptibility of *Salmonella enterica* serovar Typhi and Paratyphi in a tertiary care hospital in Dhaka, Bangladesh. *Tzu Chi Medical Journal* **26** 73-78.

Akther S, Debnath T, Chowdhury MMH 2018: Multidrug resistant *E. coli* in hospital waste water: a potential concern for public health. *Advances in Biotechnology and Microbiology* **8**.

Biyani M, Biyani R, Ushijima H, Saito M, Takamura Y, Tamiya E, Biyani M 2018: Instant enumeration of total viable bacterial counts for food quality assurance using 'DEP-On-Go'sensor. *Analytical Methods* **10** 1585-1592.

CLSI (Clinical and laboratory standard institute), 2007: *Performance standard for antimicrobial susceptibility testing*; 17th informational supplement. **27** 187.

Cowan ST, Steel KJ 1985: *Manual for the identification of medical bacteria.* Cambridge University Press. 45-60.

Hassan MM, Ahaduzzaman M, Alam M, Bari MS, Amin KB, Faruq AA 2015: Antimicrobial resistance pattern against *E. coli* and *Salmonella spp.* in environmental effluents. *International Journal of Natural Sciences* **5** 52-58.

Hocquet D, Muller A, Bertrand X 2016: What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. *Journal of HospitalInfection* **93** 395–402.

Kam KM, Luey KY, Chiu AW, Law CP, Leung SF 2007: Molecular characterization of *Salmonella enterica* Serotype *typhi* isolates by pulsed field gel electrophoresis in Hong Kong, 2000-2004. *Foodborne Pathogens and Diseases* **4** 41-49.

Khalil R, Gomaa M 2014: Evaluation of the Microbiological Quality of Conventional and Organic Leafy Greens at the Time of Purchase from Retail Markets in Alexandria, Egypt. *Polish Journal of Microbiology* **63** 237-243.

Mahmud AT, Tanim MT, Chowdhury MT, Rahaman MM, Rahman MM, Rahman MM 2019: Genetic Diversity of *Salmonella enterica* Strains Isolated from Sewage Samples of Different Hospitals in Bangladesh. *Bangladesh Journal of Microbiology* **35** 57-60.

Majumder AK, Islam N, Nite RN, Noor R 2011: Evaluation of microbiological quality of commercially available bottled water in the city of Dhaka, Bangladesh. *Stamford Journal of Microbiology* **1** 24-30.

Munshi SK, Rahman MM, Noor R 2012: Detection of virulence potential of diarrheagenic *E. coli* isolated from surface water of rivers surrounding Dhaka City. *Journal of Bangladesh Academy of Sciences* **36** 109-121.

Nahar A, Islam A, Sobur MA, Hossain MJ, Zaman SB, Rahman MB, Kabir SML, Rahman MT 2019: Detection of tetracycline resistant *E. coli* and *Salmonella spp*. in sewage, river, pond and swimming pool in Mymensingh, Bangladesh. *African Journal of Microbiology Research* **10** 30-36.

Rahman MA, Rahman AKMA, Islam MA, Alam MM 2017: Antimicrobial Resistance of *Escherichia Coli* Isolated from Milk, Beef and Chicken Meat in Bangladesh. *Bangladesh Journal of Veterinary Medicine* **15** 141-146.

Rashid M, Rakib MM, Hasan B, 2015: Antimicrobial-resistant and ESBL-producing *Escherichia coli* in different ecological niches in Bangladesh. *Infection, Ecology and Epidemiology* **5** 1-7.

Ronald M. Atlas 2015: Principles of Microbiology, 2nd edition, Published by McGraw Hill Education (India) Private Limited.

Sharmin R 2021: Prevalence of bacterial contamination in mixed vegetable salad in Dhaka city. MS thesis. Submitted to Department of Microbiology & Parasitology. Sher-e-Bangla Agricultural University.

Siddiqui MK, Khatoon N, Roy PC 2015: Untreated liquid hospital waste: potential source of multidrug resistant bacteria. *Bangladesh Journal of Microbiology* **32** 21-24.

Tesfaye H, Alemayehu H, Desta AF, Eguale T 2019: Antimicrobial susceptibility profileof selected Enterobacteriaceae in wastewater samples from health facilities, abattoir, downstream rivers and a WWTP in Addis Ababa, Ethiopia. *Antimicrobial Resistance & Infection Control* **8** 1-11.

Uddin MA 2018: Microbiological analysis of ready to eat foods collected from different places of Dhaka City, Bangladesh. *Stamford Journal of Microbiology* **8** 30-33.

Wilson ML, Mitchell M, Patrick R, Larry G, Reller L, Barth T, Michael W, Melvin P, Sybil A, Dunne W, Robert C, Welch DF 2007: Principles and Procedures for Blood Cultures; Aproved Guideline **27** 17.