

Full Length Research Paper

Detection of tetracycline resistant *E. coli* and *Salmonella* spp. in sewage, river, pond and swimming pool in Mymensingh, Bangladesh

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Antibiotic resistant bacteria and resistance genes in the environment are major health problem globally. The present study was undertaken to detect antibiotic resistant *Escherichia coli* and *Salmonella* spp. in sewage, river, pond and swimming pool. Emphasis was given on tetracycline resistant phenotype and genotype, since, tetracycline is a widely used antibiotics. Isolation and identification of antibiotic resistant *E. coli* and *Salmonella* spp. were based on morphology, staining, cultural, and biochemical properties, disk diffusion test and PCR. A total of 47 samples were collected from Mymensingh, Bangladesh. Among the 47 samples, 36 (76.59%) were found positive for *E. coli* and 42 (89.36%) for *Salmonella* spp. Phenotypically, all isolates were found resistant to tetracycline as revealed by disk diffusion test. Isolated *E. coli* were resistant to chloramphenicol (5.5%), streptomycin (16.6%) and ampicillin (97.2%) while *Salmonella* spp. to chloramphenicol (07.1%), ciprofloxacin (07.1%), streptomycin (19.1%) and ampicillin (100%). All bacterial isolates were sensitive to gentamycin. PCR result showed that 77.77 and 80.95% phenotypically tetracycline resistant *E. coli* and *Salmonella* spp. were positive for *tetA* gene. From this study it is concluded that tetracycline resistant *E. coli* and *Salmonella* spp. widely present in sewage, river, pond and swimming pool water are of great public health concern.

Key words: Environment, sewage, antibiotic resistance, *tetA*, *E. coli*, *Salmonella* spp., polymerase chain reaction, public health.

INTRODUCTION

Concern over the threat posed by antibiotic resistant bacteria and resistance genes to human health has turned greater attention also to the environmental dimensions of the problem. Only fairly recently, acknowledge has been made on the role of the environment as a source and

dissemination route for antibiotic resistance (Karkman et al., 2019). Antibiotics are used as prescribed medications to control clinical infections. They are also included in feeds for livestock and poultry as growth promoters. From human and animal, these pharmaceuticals are excreted

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from the body in our environment including water bodies and sewage through urine or feces (Lood et al., 2017).

Antibiotics in the environment act as a selective pressure to induce bacterial antimicrobial resistance (AMR). *E. coli* and *Salmonella* spp. are Gram negative enteric bacterium of the family Enterobacteriaceae and ubiquitous in the environment (Scott et al., 2002). Environment harboring antibiotic resistant bacteria (ARB) act as a source or reservoir for the spread of antibiotic resistance genes vertically into the other bacteria, thus making the situation more aggravated. In addition, from environment people can directly get exposed to this ARB or indirectly through food chain (George, 2019).

Tetracycline is one of the widely used antibiotics in veterinary and human medicine. They are also used as growth promoters for livestock and aquaculture (Li et al., 2010). Tetracycline is a broad-spectrum agents having effect against a range of Gram positive and Gram negative bacteria through inhibition of bacterial protein synthesis due to the activity of tetracycline resistance genes including *tetA-E* (Levy et al., 1999; Guillaume et al., 2000). In many cases, *tetA* is found more commonly in clinical *E. coli* than other *tet* gene family (Sengeløv et al., 2003).

ARB and their resistance genes represent a serious threat for human health since diseases caused by these resistant bacteria cannot be treated by standard therapies. Both the ARB and ARGs have been detected extensively in waste water samples globally (Bouki et al., 2013). In Bangladesh, sewage and water treatment system is not well developed. Various types of clinics and hospitals are often established near the water body in Bangladesh could be the major source of antibiotics in aquatic environments (Siddiqui et al., 2015). Even biological waste material from diagnostic laboratory and hospital are directly disposed in drain water without any treatment that are loaded with pathogenic microbes. In addition waste materials from municipal, agricultural, livestock and poultry farms are also dumped in water bodies could be contaminated with antibiotics resistant bacteria.

Recently in Bangladesh antibiotic resistant *Salmonella* spp. and *E. coli* were detected from pond water and sewage samples respectively by Mahmud et al. (2019) and Sobur et al. (2019). Previously Zahid et al. (2009) reported the occurrence of multidrug resistance (MDR) *E. coli* in surface water in Bangladesh. However, not molecular based adequate surveillance data are available in Bangladesh on the occurrence of tetracycline resistant *E. coli* and *Salmonella* spp. in sewage, river, pond and swimming pool water. These surveillance data on AMR are crucial to support the National Action Plan of AMR of Bangladesh Government to take necessary steps to tackle the AMR related hazards. Therefore, the present study aimed to explore the presence and rate of the public health important tetracycline resistant *E. coli* and *Salmonella* spp. in untreated sewage, river, pond and

swimming pool water samples.

MATERIAL AND METHODS

Collection of samples

Aseptically, sampling was carried out over the period of June to July, 2018 on random basis. A total of 47 water samples were collected from different areas of Mymensingh, Bangladesh including 20, 4, 20 and 3 from sewage, river, pond and swimming pool water, respectively. From each case 250 ml water samples were collected aseptically in sterile glass bottle labeled properly and transported to the laboratory maintaining cool chain for immediate processing.

Isolation and identification of *E. coli* and *Salmonella* spp.

Isolation and identification of *E. coli* and *Salmonella* spp. were carried out based on initial culture in nutrient broth (6 h at 37°C aerobically) followed plating on Eosine Methylene Blue (EMB) agar and Xylose Lysine Dextrose (XLD) agar plates (Hi Media, India) respectively. Culture plates were aerobically incubated at 37°C for 24 h followed by observing the cultural characteristics, morphology, staining, and biochemical test as described by Bergey et al. (1974). Isolation of *E. coli* and *Salmonella* spp. were confirmed by PCR targeting 16S rRNA gene and *invA* genes, respectively as described subsequently.

Extraction of genomic DNA

Genomic DNA for the PCR was extracted by boiling method as described previously by Mahmud et al. (2018). In brief, initially 100 µl of deionized water was taken into an Eppendorf tube. A pure bacterial colony of *E. coli* or *Salmonella* spp. from overnight culture on EMB or XLD agar plate at 37°C was gently mixed with deionized water. The tube was then transferred into boiling water and boiled for 10 min, then immediately transferred into ice for cold shock for about 10 min, and finally centrifuged at 10,000 rpm for 10 min. Supernatant from each tube was collected and used as template DNA for PCR. The extracted DNA was stored at -20°C until use.

E. coli and *Salmonella* spp. specific PCR

Primers and protocol used for the detection of *E. coli* and *Salmonella* spp. is listed in Table 1. All the PCR were done in a final 25 µl reaction with 12.5 µl master mixture 2X (Promega, USA), 2 µl genomic DNA (30 ng), 1 µl each primer (10 picomol) and 8.5 µl nuclease free water.

Thermal profile for PCR

Thermal condition was consisted of initial denaturation at 95°C for 5 min followed by 30 cycles each of denaturation at 94°C for 30 s, optimal annealing temperature for each primer set (Table 1), extension at 72°C for 1 min and final extension at 72°C for 10 min.

Visualization of amplified products

Amplified PCR products were analyzed by electrophoresis in 1.5% agarose gel. Ethidium bromide was used to stain product which were visualized under ultraviolet trans-illuminator (Biometra,

Table 1. Primers used for the detection of *E. coli*, *Salmonella* spp. and *tetA* gene.

Target gene	Primer sequence (5'-3')	Product size (bp)	Annealing temperature (°C)	References
<i>invA</i>	F: ATCAGTACCAGTCGTCTTATCTTGAT R:TCTGTTTACCGGGCATAACCAT	211		Shanmugasundaram et al. (2009)
EC 16S rRNA	F: GACCTCGGTTTAGTTCACAGA R:CACACGCTGACGCTGACCA	585		Candrian et al. (1991)
<i>tetA</i>	F: GGTTCACTCGAACGACGTCA R: CTGTCCGACAAGTTGCATGA	577		Woodford and Livermore (2009)

Table 2. Isolation of *E. coli* in sewage, river, pond and swimming pool.

Source of sample	Number of sample	<i>E. coli</i>		<i>Salmonella</i> spp.	
		Number of positive samples	Occurrence (percentage)	Number of positive samples	Occurrence (percentage)
Sewage	20	17	85	19	95
River	04	03	75	04	100
Pond	20	15	75	18	90
Swimming pool	03	01	33.33	01	33.33
Total	47	36	76.59	42	89.36

Germany). 100 bp DNA ladder (Promega, USA) was used as molecular weight marker.

In vitro antibiotic sensitivity test

Six commonly prescribed antibiotics (HiMedia, India) namely chloramphenicol (10 µg), ciprofloxacin (5 µg), gentamicin (10 µg), tetracycline (30 µg), streptomycin (10 µg) and ampicillin (2 µg) were selected for the sensitivity test. Antibigram were done by disk diffusion method using Mueller Hinton (HiMedia, India) agar media as described by Mamun et al. (2017). A McFarland 0.5 standard was maintained for each culture suspension of bacterial isolates. The results of the test were recorded as sensitive, intermediately sensitive, or resistant by the recommendations of CLSI (2016).

Molecular detection of the *tetA* gene

Isolated *E. coli* and *Salmonella* spp. that were found phenotypically resistant to tetracycline were further screened to detect tetracycline resistance gene, *tetA* by PCR using the primers and protocol as presented in Table 1. All the PCR were done as stated previously. Thermal condition consisted initial denaturation at 95°C for 5 min followed by 30 cycles each of 95°C for 60 s, 57°C for 60 s, 72°C for 1 min and final extension at 72°C for 10 min. Agarose gel 1.5% was used to analyzed and amplified the PCR product by electrophoresis.

RESULTS

Isolation of *E. coli* and *Salmonella* spp.

Among the 47 samples collected, 36 (76.59%) were found positive for *E. coli*. On sample basis, the highest occurrence was in sewage (85%) and lowest in swimming

pool (33.33%; Table 2). On the other hand among the 47 samples, 42 (89.36%) were found positive for *Salmonella* spp. Occurrence of *Salmonella* spp. was highest in river and lowest in swimming pool (Table 2).

Antibiogram profile

All the isolates were subjected to antibiogram study. Phenotypically among 36 *E. coli* isolates, two were found resistant to chloramphenicol, six to streptomycin, 35 to ampicillin and all to tetracycline (Table 3). On the other hand phenotypically among the 42 *Salmonella* spp. isolates, three were found resistant to chloramphenicol, three to ciprofloxacin, eight to streptomycin, and all to tetracycline and ampicillin. Notable finding is that all the isolated *E. coli* and *Salmonella* spp. were found resistant to tetracycline, while all were sensitive to gentamicin.

Detection of *tetA* gene

Tetracycline resistant phenotypes were screened for the detection of *tetA* gene by PCR (Figure 1). Among the 36 *E. coli* 28 (77.80%) isolates were found positive for *tetA* gene (Table 4). In case of *Salmonella* spp. among the 42 isolates, 34 (80.90%) were found positive for *tetA* gene.

DISCUSSION

Because of the rapid emergence and spread of antibiotic

Table 3. *In vitro* antibiotic sensitivity test of the isolated *E. coli* and *Salmonella* spp.

Name of antibiotic	Isolated bacteria	Sensitive	Intermediate	Resistant
Gentamicin	E (36)	33 (91.6%)	03 (08.3%)	0 (0.00%)
	S (42)	34 (80.9%)	08 (19.1%)	0 (0.00%)
Chloramphenicol	E (36)	26 (72.2%)	08 (22.2%)	02 (05.5%)
	S (42)	30 (71.4%)	09 (21.45%)	03 (07.1%)
Ciprofloxacin	E (36)	24 (66.6%)	12 (33.3%)	0 (0.00%)
	S (42)	31 (73.8%)	08 (19.1%)	03 (07.1%)
Streptomycin	E (36)	23 (63.8%)	07 (19.4%)	06 (16.6%)
	S (42)	16 (38.1%)	18 (42.8%)	08 (19.1%)
Ampicillin	E (36)	0 (0.00%)	01 (02.7%)	35 (97.2%)
	S (42)	0 (0.00%)	0 (0.00%)	42 (100%)
Tetracycline	E (36)	0 (0.00%)	0 (0.00%)	36 (100%)
	S (42)	06 (14.2%)	0 (0.00%)	42 (100%)

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

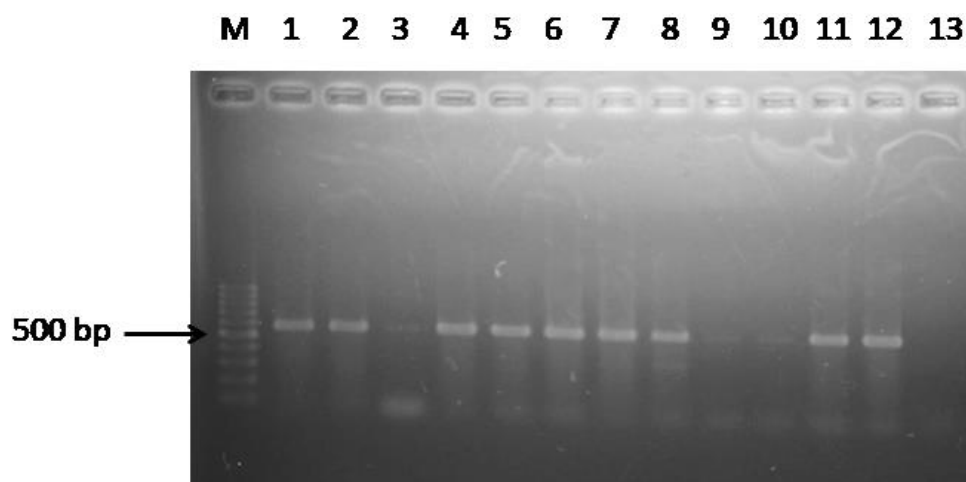


Figure 1. PCR amplification of 577 bp amplicon of *tetA* gene. Lanes 1-6: *E. coli*, lanes 7-11: *Salmonella* spp. M 100 bp. Lane 12: positive control, lane 13: negative control.

Table 4. Distribution of *tetA* gene among the isolated *E. coli* and *Salmonella* spp.

Source of sample	Tetracycline resistant <i>E. coli</i>		Tetracycline resistant <i>Salmonella</i> spp.	
	No. of phenotype tested	<i>tetA</i> positive	No. of phenotype tested	<i>tetA</i> positive
Sewage	17	14/17 (82.3%)	19	17/19 (89.4%)
River	03	2/3 (66.6%)	04	1/4 (25%)
Pond	15	11/15 (73.3%)	18	15/18 (83.33%)
Swimming pool	01	1/1 (100%)	01	1/1 (100%)
Total	36	28 (77.77%)	42	34 (80.95%)

resistant bacteria and their resistance genes among humans, animals and the environment at global scale, antibiotic resistance is now considered as a one health challenge. Environment is a major source for antibiotic

resistant bacteria that are of great public health concern. Most of the researches on AMR focused on human and animal, and there is a lack in AMR situation in the environment in LMICs such as in Bangladesh. In this

study, the occurrence of antibiotic resistant *E. coli* and *Salmonella* spp. in sewage, river, pond and swimming pool in Mymensingh, Bangladesh with molecular level was investigated.

In this study *E. coli* and *Salmonella* spp. were found to be widely distributed in the environmental samples analyzed. The overall occurrence of *E. coli* and *Salmonella* spp. were 76.59 and 89.36%, respectively. One of the most striking finding of this study is that all the isolates of *E. coli* and *Salmonella* spp. were found resistant to tetracycline phenotypically. This observation is further supported by the detection of *tetA* gene. Resistance against tetracycline is usually associated with *tet* gene family. Tetracycline is a widely used antibiotic (Hassan et al., 2015). Long time wide spread use of tetracycline in veterinary and human medicine could be lined with these observed resistant against tetracycline. Peak et al. (2007) and Huang et al. (2019) also found tetracycline resistance genes in various types of waste water and sewage. It is also important to note that few isolated *E. coli* and *Salmonella* spp. in this study were found to be MDR in nature for example, resistant against tetracycline, ampicillin and streptomycin. Rashid et al. (2015) earlier reported the presence of MDR *E. coli* in various aquatic sources in Bangladesh.

It is not uncommon to detect these ARB in environmental samples as evident from the recent work of Divya and Hatha (2019), Proia et al. (2019) and Liu et al. (2018), who also detected antibiotic resistant *E. coli* and *Salmonella* spp. in various environmental samples including tropical estuarine water, waste water, sewage etc. In this study, tetracycline resistant *E. coli* and *Salmonella* spp. in water collected from sewage, river, pond and swimming pool were detected. Wei et al. (2018) detected several member of Enterobacteriaceae in swimming pool water in Guangzhou, China and in Imo river water in Nigeria (Ihejirika et al., 2011). Contamination of surface water with biological waste including fecal materials could be associated with the occurrence of these resistance bacteria in these environmental samples.

In Bangladesh, Zahid et al. (2009) carried out an investigation on the prevalence of multiple ARB and their chromosomal determinants in surface water. From 147 samples, they isolated 103 bacterial species of which 65% were *E. coli* including isolates resistant to tetracycline. While Siddiqui et al. (2015) showed presence of antibiotic resistant *Salmonella* spp. in hospital waste and many of which eventually ended up in the sewage in Bangladesh, the present study findings support both of these earlier observations.

E. coli and *Salmonella* spp. are enteric bacteria. Although not all the strains of *E. coli* and *Salmonella* spp. are pathogenic in nature, some strains are capable of causing serious illness in animal and human including enteritis. Both of them are also zoonotic in nature (Vasco et al., 2016). Detection of antibiotic resistant *E. coli* and

Salmonella spp. in sewage, river, pond and swimming pool as evident in this study are very alarming from the public health point of view. Antibiotic resistance is a global health problem. Disease caused by ARB are very difficult to treat, needs special attention and expensive to treat. Human can easily get exposed to these resistant strains from sewage, river, pond and swimming pool. Moreover, occurrence of antibiotic resistant bacteria in the environmental samples observed in this study is an indication of serious environmental pollution and hazard. Many of the resistant genes are mobile in nature. Environment contaminated with resistant bacteria and resistance genes act as source or reservoir for AMR that can easily transmit to other bacterial species.

There are a number of limitations associated with this study. Although tetracycline resistant *E. coli* and *Salmonella* spp. in various environmental samples have been detected here, not much samples were analyzed. In addition, the virulence properties of these isolates were also not investigated. Molecular basis of other resistant phenotypes were not focused. More detail study focusing on these limitations will provide a better understanding of AMR in environmental samples.

Conclusion

Environmental contamination is a global health challenge of the 21st century. In this study a wide spread occurrence of antibiotic resistant *E. coli* and *Salmonella* spp. in sewage, river, pond and swimming pool including *tetA* gene responsible for resistance against tetracycline were detected. The presence of these resistant bacteria in this environment is of great public health concern. Many strains of *E. coli* and *Salmonella* spp. are pathogenic in nature and there is potentiality for transmission of these pathogens to human from the contaminated environment. Disease caused by resistant isolates is difficult to treat. Food chain and animal are also at the risk of contamination. It is suggested that establishing active surveillance system across the nation for detection of ARB and ARGs in various environmental samples will assist in reducing hazards associated with AMR on animals and humans.

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CONFLICT OF INTEREST

The authors have not declared any conflict of interests.

REFERENCES

- Bergey DH, Buchanan RE, Gibbons NE (1974). American Society for Microbiology Bergey's manual of determinative bacteriology. Baltimore: Williams and Wilkins Co.
- Bouki C, Venieri D, Diamadopoulos E (2013). Detection and fate of antibiotic resistant bacteria in wastewater treatment plants: A review. *Ecotoxicology Environment and Safety* 91:1-9.
- Candrian U, Furrer B, Hofelein C, Meyer R, Jermini M, Luthy J (1991). Detection of *Escherichia coli* and identification of enterotoxigenic strains by primer-directed enzymatic amplification of specific sequences. *International Journal of Food Microbiology* 12:339-352.
- Clinical and Laboratory Standards Institute (CLSI) (2016). Performance standards for antimicrobial susceptibility testing. 26th edition, CLSI supplement M100s. Clinical and Laboratory Standards Institute, Wayne, Pennsylvania.
- Divya SP, Hatha AAM (2019). Screening of tropical estuarine water in south-west coast of India reveals emergence of ARGs-harboring hypervirulent *Escherichia coli* of global significance. *International Journal of Hygiene and Environmental Health* 222(2):235-248.
- George A (2019). Antimicrobial Resistance (AMR) in the Food Chain: Trade, Health and Codex. *Tropical medicine and infectious disease* 4(1):54.
- Guillaume G, Verbrugge D, Chasseur-Libotte ML, Moens W, Collard JM (2000). PCR typing of tetracycline resistance determinants (*Tet A-E*) in *Salmonella enterica* serotype Hadar and in the microbial community of activated sledges from hospital and urban wastewater treatment facilities in Belgium. *FEMS Microbiology Ecology* 32:77-85.
- 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 Science* 5(2):52-58.
- Huang YH, Liu Y, Du PP, Zeng LJ, Mo CH, Li YW, Lü H, Cai QY (2019). Occurrence and distribution of antibiotics and antibiotic resistant genes in water and sediments of urban rivers with black-odor water in Guangzhou, South China. *Science of Total Environment* 670:170-180.
- Ihejirika CE, Ogbulie JN, Nwabueze RN, Orji JC, Ihejirika OC, Adieze, IE, Ibe IJ (2011). Seasonal influences on the distribution of bacterial pathogens and waterborne diseases transmission potentials of Imo river, Nigeria. *The Journal of Biological Research* 3:163-172.
- Karkman A, Pärnänen K, Larsson DGJ (2019). Fecal pollution can explain antibiotic resistance gene abundances in anthropogenically impacted environments. *Nature Communications* 10(1):80.
- Levy SB, McMurry LM, Barbosa TM, Burdett V, Courvalin P, Hillen W, Roberts MC, Rood R, Taylor DE (1999). Nomenclature for new tetracycline resistance determinants. *Antimicrobial Agents and Chemotherapy* 43:1523-1524.
- Li D, Yu T, Zhang Y, Yang M, Li Z, Liu M, Qi R (2010). Antibiotic resistance characteristics of environmental bacteria from an oxytetracycline production wastewater treatment plant and the receiving river. *Applied and Environmental Microbiology* 76(11):3444-3451.
- Liu H, Whitehouse CA, Li B (2018). Presence and persistence of *Salmonella* in Water: The Impact on microbial quality of water and food safety. *Frontiers in Public Health* 6:159.
- Lood R, Ertürk G, Mattiasson B (2017). Revisiting antibiotic resistance spreading in wastewater treatment plants – bacteriophages as a much neglected potential transmission vehicle. *Frontiers in Microbiology* 8:2298.
- 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(1):57-60.
- Mahmud S, Nazir KHMNH, Rahman MT (2018). Prevalence and molecular detection of fluoroquinolone-resistant genes (*qnrA* and *qnrS*) in *Escherichia coli* isolated from healthy broiler chickens. *Veterinary World* 11(12):1720-1724.
- Mamun MM, Hassan J, Nazir KHMNH, Islam MA, Zesmin K, Rahman MB, Rahman MT (2017). Prevalence and molecular detection of quinolone-resistant *E. coli* in rectal swab of apparently healthy cattle in Bangladesh. *International Journal of Tropical Disease and Health* 24(2):1-7.
- Peak N, Knapp CW, Yang RK, Hanfelt MM, Smith MS, Aga DS, Graham DW (2007). Abundance of six tetracycline resistance genes in wastewater lagoons at cattle feedlots with different antibiotic use strategies. *Environmental Microbiology* 9(1):143-151.
- Proia L, Anzil A, Subirats J, Borrego C, Farré M, Llorca M, Balcázar JL, Servais P (2019). Antibiotic resistance along an urban river impacted by treated wastewaters. *Science of Total Environment* 628:453-666.
- 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):26712.
- Scott TM, Rose JB, Jenkins TM, Farrah SR, Lukasik J (2002). Microbial source tracking: current methodology and future directions. *Applied and Environmental Microbiology* 68(12):5796-5803.
- Sengeløv G, Halling-Sørensen B, Aarestrup FM (2003). Susceptibility of *Escherichia coli* and *Enterococcus faecium* isolated from pigs and broiler chickens to tetracycline degradation products and distribution of tetracycline resistance determinants in *E. coli* from food animals. *Veterinary Microbiology* 29:95(1-2):91-101.
- Shanmugasundaram M, Radhika M, Murali HS, Batra HV (2009). Detection of *Salmonella enterica* serovar Typhimurium by selective amplification of *fliC*, *fliB*, *iroB*, *invA*, *rfbJ*, *STM2755*, *STM4497* genes by polymerase chain reaction in a monoplex and multiplex format. *World Journal of Microbiology and Biotechnology* 25(8):1385-1394.
- Siddiqui MK, Khatoun N, Roy PC (2015). Untreated liquid hospital waste: potential source of multidrug resistant bacteria. *Bangladesh Journal of Microbiology* 32:21-24.
- Sobur MA, Haque ZF, Nahar A, Zaman SB, Rahman MT (2019). Emergence of colistin resistant *E. coli* in poultry, house flies and pond water in Mymensingh, Bangladesh. *Journal of Advanced Veterinary and Animal Research* 6(1):50-53.
- Vasco K, Graham JP, Trueba G (2016). Detection of zoonotic enteropathogens in children and domestic animals in a semirural community in Ecuador. *Applied and Environmental Microbiology* 82:4218-4224.
- Wei X, Li J, Hou S, Xu C, Zhang H, Atwill ER, Li X, Yang Z, Chen S (2018). Assessment of microbiological safety of water in public swimming pools in Guangzhou, China. *International Journal of Environmental Research and Public Health*. 15(7):1-12
- Woodford N, Livermore DM (2009). Infections caused by Gram-positive bacteria: a review of the global challenge. *Journal of Infection* 59(1):S4-S16.
- Zahid HM, Mahal Z, Chowdhury MR (2009). Prevalence of multiple antibiotic resistant bacteria and chromosomal determinants in surface water of Bangladesh. *African Journal of Biotechnology* 8(2):148-154.