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A study on some aspects of bacteriological qualities of hospital wastewater in and around Guwahati city

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Abstract
The bacteriological qualities of wastewater originating from both veterinary and human healthcare establishments in and around Guwahati city were investigated to assess the influence of the hospital wastewater in the receiving environment. In the present study, 10 sampling locations were selected and samples were collected twice from each location for a period of 5 months. A total of 100 wastewater samples were analysed for different bacteriological qualities. Altogether a total of 140 bacterial isolates (E. coli, Salmonella, Streptococcus, Staphylococcus and Klebsiella) were recovered, of which 55 (39.29%) and 85(60.71%) isolates were from the wastewater of veterinary healthcare establishments and human hospitals respectively during the study period. Among the various bacteria isolated Escherichia coli was the most predominant (53.57%), followed by Salmonella (21.42%), Streptococcus (13.57%), Staphylococcus (5.71%) and Klebsiella (5.71%). The results of antibiotic sensitivity tests of these isolates towards 8 antimicrobial drugs (Ampicillin, Gentamicin, Ceftriaxone, Norfloxacin, Oxytetracycline, Streptomycin, Penicillin-G and Colistin) revealed that E. coli and Salmonella showed highest sensitivity towards Gentamicin (100%). On the other hand E. coli, Salmonella and Klebsiella showed no sensitivity towards Ampicillin and Penicillin-G. For Staphylococcus highest sensitivity was towards Norfloxacin and Ceftriaxone (87.7%) and no sensitivity towards Gentamicin. Thus, the present study based on the sampling of wastewater in and around Guwahati city indicated that the wastewater discharged by these locations lack adequate treatment and management process. Therefore, there is significant impact on health and contamination of the receiving environment.

Keywords: Aspects, bacteriological, qualities, wastewater, veterinary

1. Introduction
Wastewater is attributed to any water whose quality has been decreased by irregular efforts of human. Wastewater includes liquid waste generated from agriculture, domestic means, industries, human excretion, commercial sectors, pharmaceuticals, healthcare units) of water which quality has been deteriorated under anthropogenic influences (Buelow *et al.* 2017) [5]. Hospital effluent is referred to as wastewater from hospitals or healthcare centres, biological or non-biological that is discarded and not intended for further use (Oyeleke *et al*. 2008) [15]. With the public's increasing demands on healthcare standards, advancements in medical technology and rapid development in the pharmaceutical sciences, the number and complexity of hospital activities have increased and thus have led to more negative impacts through the increase in wastewater production by hospitals. Despite the growing concern over clinical institutional waste management, scanty attention has been paid to wastewater generated from hospitals, clinical research laboratories and health care institutions.
Potable water of good quality is essential for life. Human activities interfere in many ways with natural water cycle and affect the society-water relationship. Constantly increasing human population and its expectations regarding the standard of living increase demands on exploitation of existing resources including water (Chowdhury, 2013) [6]. Monitoring of safety of water sources is based on determination of parameters that indicate pollution caused by sewage, animal excrements, storage of waste, animal manure and artificial fertilizers (Sasakova *et al*. 2013 and Fridrich *et al*. 2014) [20, 10]. WHO [21] reported that, 80% of all diseases in the developing countries results from contaminated water.
Hospitals use a variety of chemical substances such as pharmaceuticals, radio nucleotides, solvents, disinfectants for medical purposes as diagnostics, disinfections and research. Unused medications also are sometimes disposed of hospital drain. Due to laboratory and research activities or medicine excretion into wastewater, hospitals may represent an incontestable

release source of many toxic substances in the aquatic environment (Jolibois *et al*. 2002) [13]. The discharge of wastewater depends upon the capacity of hospital and generally water varying from 400 to 1200 L/day/bed is consumed by the hospitals (Akin, 2016) [1]. The absence of specific pre-treatment technologies for hospital wastewater also increased the frequency of gastroenteric viruses in aquatic bodies (Ibrahim *et al*. 2018) [12].

Hospital wastewater, bearing the contents of infectious biological and hazardous chemical substances, does not just pose health hazards to human beings, but is also capable of causing irreversible destructions and tremendous damages to the nature. Antibiotic resistant genes and antibiotic resistant bacteria have been detected in wastewater samples (Reinthaler *et al*. 2003; Pruden *et al*. 2006; Auerbach *et al*. 2007 and Brooks *et al.* 2007) [18, 17, 3, 4]. Improper disposal of waste from hospitals and public health care units poses greater threat than the original diseases themselves due to the presence of concentrated forms of numerous risks including pathogenic and antibiotic resistant microorganisms.

In India, municipal sewage treatment plants are not so common and there is a tendency of disposal of wastewater directly into the community sewer system without any treatment (Gautam *et al*. 2007) [11]. The community sewer system finally ends in the water bodies like lake, river and ocean without tertiary treatment. The scenario in the Northeastern region is also not different. The low efficacy of the hospital sewage treatment may contribute to the dissemination of multidrug resistant bacteria from hospital compartments to the environment. Indeed hospital wastewater may have an adverse impact on environmental and human health; therefore, the proper management of hospital wastewater quality and quantity is needed (Jolibois and Guerbet, 2006) [14]. Looking at the possible danger posed by hospital wastewaters, this work has been done to study the wastewater management systems in different human and veterinary healthcare establishments and to assess the bacteriological properties of wastewater from human and veterinary healthcare establishments.

2. Materials and Methods

2.1 Existing wastewater management system:

Managemental aspects regarding wastewater disposal, treatment plant efficiency, public awareness and hospital staff training were studied through spot verification. Questionnaires were provided to the hospital staffs to get the information regarding wastewater and sludge treatment procedures and disposal systems in their respective hospitals.

2.2 Collection of sample and sampling locations

Collection, storage and preservation of samples were done as per methods described by American Public Health Association (APHA, 1998) [2]. The wastewater samples were collected twice in a month from each location for a period of 5 months in pre-cleaned polyethylene (plastic) containers starting from January to May 2019. The containers were pre-cleaned and rinsed with distilled water several times and dried thoroughly before use. For bacteriological analysis, wastewater samples were collected in sterilized glass bottles. The containers in all case were filled and tightly stoppered to avoid contact with air or to prevent agitation during transport. The locations for sampling were selected *viz*. Teaching Veterinary Clinical complex, Pet and vet (Zoo road Tiniali), Chenikhutti veterinary clinic, JBF (Beltola), Pet and Vet (Geeta Mandir) GMC hospital, GNRC hospital (Sixth mile), Saraighat hospital, Dispur hospital and GNRC (Dispur).

2.3 Storage and preservation of samples

Storage and preservation of samples were done following standard procedure (APHA, 1998) [2].The parameters, *viz*., temperature and turbidity were measured at the time of collection of samples. The samples were also brought immediately to the laboratories for the analysis of bacteriological parameters selected for the present study.

2.4 Isolation of bacteria

a. Media

All the collected samples were subjected for bacteriological investigation. Samples were inoculated into blood agar (5-10%) media by streak plate technique as per the method described by Edwards and Ewing (1972) [8] and Cruickhank *et al*. (1975) [7]. Plates were incubated aerobically at 37°C for 24 hrs. After incubation, colony morphologies were studied and purification of the colonies was done by inoculating a single colony into the following nutrient media according to the type of bacteria-

(i) Brain Heart Infusion (BHI) Agar. (ii) Mac Conkey's Lactose Agar (MLA) (iii) Eosin Methylene Blue (EMB) Agar and (iv) Brilliant Green Agar (BGA)

b. Maintenance of cultures

After purification the bacterial colonies were picked up and streaked onto NA slants, which were further incubated at 37 °C for 24hrs. These slants were then sealed with paraffin and preserved at 4 °C. The organisms were subcultured on fresh NA slants at every six weeks interval to maintain their viability.

c. Characterization and identification of the organism:

Characterization and identification of the organism were made as per Edwards and Ewing, (1972) [8] and Cruickshank *et al*. (1975) [7] on the basis of the following:

1. Colony Morphology
2. Cell morphology and staining characteristics
3. Biochemical tests

(a) Different sugar fermentation (Glucose/ lactose) (b) Indole test (c) Methyl Red test (d) Voges Proskauer test (e) Citrate utilization test (f) Acid butt test and H_2S production test

2.5 Antimicrobial sensitivity test

Antimicrobial susceptibility test was carried out by disc diffusion technique using commercially available antimicrobial agents. The sensitivity testing discs were obtained from Hi-Media Laboratories, Mumbai, India.

a. Sensitivity test procedure

About 2ml of the inoculums was poured on the Mueller Hinton Agar plates and was spread uniformly on the surface of the plates. The plates were kept undisturbed for about 5 minutes. The excess inoculum was sucked out from the plate with the help of a sterile Pasteur pipette and the antimicrobial discs were placed gently on the agar surface maintaining adequate distance among them. Each disc were gently pressed down with a pair of sterile forceps to ensure complete contact with the medium. The plates were then incubated aerobically at 37 °C for 18-24 hrs in inverted position.

b. Reading and interpretation

After 24 hrs of incubation, the zones of inhibition were measured following the standard and the results were interpreted by using the 'zone size interpretative table' provided by the manufacturer of the discs.

3. Results and Discussion

3.1 Existing wastewater management system

In the present study, management systems of wastewater produced in the veterinary and human hospitals were evaluated through questionnaires. This survey showed that there were two types of sewage disposal system, 50% of hospitals use septic tanks for wastewater disposal and 50% dispose directly into the sewer. Findings showed that 50% of surveyed hospitals practice regular inspection and repair of leaks and 30% regulate wastewater treatment process regularly. 60% of the surveyed hospitals conduct awareness programmes regarding environmental safety in their hospitals (Table 1).

Table 1: Frequency distribution of respondents based on the response of existing wastewater management system

	Questionnaires	Frequency	
		Yes	No
1	Does your wastewater go to septic tank?	5(50.00)	5(50.00)
2	Does your wastewater go to sewer?	5(50.00)	5(50.00)
3	Does you sample your wastewater discharge?	0.00	10(100.00)
4	Do you have a discharge permit or authorization to discharge a pollutant permit number?	6(60.00)	4(40.00)
5	Do you have a diagram of your sewer discharge?	0.00	10(100.00)
6	Do you implement any water conservation program?	0.00	10(100.00)
7	Do you practice regular inspection and repair of leaks?	5(50.00)	5(50.00)
8	Do you discharge dangerous wastes down the stream?	0	10(100.00)
9	Do you regulate wastewater treatment process efficiently?	3(30.00)	7(70.00)
10	Any awareness programmes?	6(60.00)	4(40.00)

3.2 Isolation and identification of different aerobic bacteria

Isolation and identification: In the present study, a total of 100 samples were subjected to bacteriological examination. All the samples were found to be bacteriologically positive yielding one or more types of bacteria. Details of the prevalence and isolation of bacteria from different sewage samples were depicted in the Table 2. Altogether a total of 140 isolates were recovered during the study, of which 55 (39.29%) isolates were obtained from the wastewater of veterinary clinics and the remaining 85 (60.71%) were from the wastewater of human hospitals. The number of different aerobic bacterial isolates recovered from wastewater of both veterinary clinics and human hospitals were shown in Table 3. Among the various bacteria isolated E. coli was the most predominant (53.57%), followed by Salmonella (21.42%), Streptococcus (13.57%), Staphylococcus (5.71%) and Klebsiella (5.71%). The present findings corroborated with the findings of Pradhan and Mishra (2010) [16] and Elmanama *et al*. (2006) [9]. Altogether 113 isolates were identified as gram-negative bacteria on the basis of staining reaction. These were subjected to different biochemical tests as shown in Table 4. The bacteria identified included *E. coli* (75), Salmonella (30) and Klebsiella (8).

Table 2: Shows the number of different aerobic bacterial isolates from sewage

Source of sample	No. of sample Tested	No. of samples bacteriologically positive	No. and type of bacterial species isolated
Veterinary Clinics	50	50	*E. coli (32)*
			Salmonella (13)
			Klebsiella (2)
			Streptococcus (4)
			Staphylococcus (4)
Hospitals	50	50	*E.coli (43)*
			Salmonella (17)
			Klebsiella(6)
			Streptococcus (15)
			Staphylococcus (4)

Table 3: Antibiotic sensitivity tests of different isolates from healthcare wastewater

Source	Name of isolated bacteria	Number of isolate tested	Number of isolate sensitive to							
			AM	CTR	GEN	NX	O	P	S	CL
Human hospitals and Veterinary clinics	*E. coli*	75	0 0.00	10 13.33	75 100.00	65 86.67	22 29.33	0 0.00	21 28.00	35 46.67
	Salmonella	30	0 0.00	20 66.67	30 100	22 73.33	15 50.00	0 0.00	22 73.33	20 66.67
	Streptococcuss	19	7 36.84	12 63.17	18 94.73	16 84.21	10 52.63	6 31.57	17 89.47	17 89.47
	Staphylococcus	8	2 25.00	7 87.7	0 0.00	7 87.7	5 62.5	6 75.00	2 25.00	5 62.5
	Klebsiella	8	0 0.00	5 71.43	5 71.43	6 85.71	4 57.14	0 0.00	4 57.14	3 42.86
	TOTAL	140								

AM: Ampicillin, CTR: Ceftriaxone, GEN: Gentamicin, NX: Norfloxacin, O:oxytetracycline, P:Penicillin-G, S:Streptomycin, CL: Colistin

Table 4: Biochemical characteristics of different aerobic bacterial isolates from sewage

Biochemical characteristics	Sugar fermentation		Coagulase test	Catalase test	Citrate test	Indole test	Methyl red test	Voges proskauer test	H2S test	Acid butt test
	Glucose	Lactose								
E. *coli*	75	75	-	-	0	75	75	0	0	-
Salmonella	-	-	-	-	29	0	29	0	29	29
Staphylococcus	-	-	8	8	-	-	-	-	-	-
Streptococcuss	-	-	0	0	-	-	-	-	-	-
Klebsiella	8	8	-	-	8	0	0	8	-	-

All the isolates were identified on basis of morphology, staining and biochemical characteristics. Strains of E.coli were found to ferment glucose and lactose. All the isolates were observed to be positive MR (Methyl Red). Citrate utilization test and Voges proskauer test were negative for all the isolates. The Salmonella atrains showed positive reactions to MR, H_2S, acid butt test and citrate utilization test but showed negative tests for indole test.

Whereas strains of Klebsiella were positive for citrate utilization, VP test, sugar fermentation test (lactose and glucose) but negative for indole and MR tests. The remaining 27 isolates identified as Gram-positive bacteria on the basis of staining reaction were also subjected to different biochemical characteristics. A total of 19 and 8 isolates showed morphological, staining and biochemical characteristics typical of the bacteria Streptococcus and Staphylococcus respectively.

3.3 Antibiotic sensitivity tests

E.coli isolates showed high degree of sensitivity towards Gentamicin (100%) followed by Norfloxacin (86.67%), Colistin(46.67%) and least sensitivity towards Oxytetracycline (29.33%), Streptomycin (28%) and Ceftriaxone (13.33%). The isolates showed no sensitivity towards Ampicillin. Salmonella isolates were highly sensitive towards Gentamicin (100%), Norfloxacin and Streptomycin (both 73.33%), Ceftriaxone and Colistin (both 66.67%), Oxytetracycline (50%) but showed no sensitivity towards Ampicillin and Penicillin-G. High sensitivity to Gentamycin (94.73%) was observed for Streptococcus isolates followed by Streptomycin, Colistin (89.47% both), Norfloxacin (84.71%), Ceftriaxone (63.17%), Oxytetracycline (52.63%) and least sensitivity were shown to Ampicillin (36.84%) and Penicillin-G (31.57%).

For Staphylococcal isolates, highest sensitivity were shown towards Norfloxacin and

Ceftriaxone (87.7% both) followed by Penicillin-G (75%), Colistin and Oxytetracycline (62.5% both) and least sensitivity was shown towards Ampicillin and Streptomycin (25% both) but no sensitivity towards Gentamicin.

Klebsiella isolates were highly sensitive towards Norfloxacin (85.71%), Ceftriaxone and Gentamicin (71.43% both), streptomycin and Oxytetracycline (57.14% both) and least towards Colistin (42.86%). The isolates showed no sensitivity towards Ampicillin and Penicillin-G.

The hospital waste water contain various chemicals like pharmaceuticals radio-nucleotides, disinfectants, excreted non metabolized drugs by the patients. Unused medications were discarded into the hospital's sewage disposal system and later into the public sewage system (Resende *et al*. 2009) [19]. Moreover, it also contains blood, pathological wastes, hospital cafeteria wastes, thus becoming the nutrient rich culturing and growth of pathogens including multiple drug resistant bacteria.

The present study, based on the bacteriological parameters of hospital wastewater analysis in and around Guwahati city indicated absence of any kind of wastewater management system or low efficacy of hospital treatment process, The wastewater produced are directly disposed off in the environment or the public sewer.

4. Conclusion

It was observed that hospital waste water samples analyzed for bacteriological parameters were much above the WHO [21] limits for hospital waste water. Therefore, this study indicated the need of effective enforcement of regulations associated with healthcare liquid waste management practice both in veterinary and human healthcare establishment to decrease the risk of disseminating pathogenic and multiple drug resistant microorganisms in the community for the safeguard of peoples health.

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6. References

1. Akin BS. Contaminant properties of hospital clinical laboratory wastewater: a physiochemical and microbiological assessment. Journal of Environmental Protection 2016;7(5):635.
2. APHA. Standard method for examination of water and wastewater 20th Ed. APHA-AWWA-WEF, Washington American Public Health Association, 1998.
3. Auerbach EA, Seyfried EE, McMahon KD. Tetracycline resistance genes in activated sludge wastewater treatment plants. Water Research 2007;41:1143 -1151.
4. Brooks JP, Maxwell SL, Rensing C, Gerba CP, Pepper, IL. Occurrence of antibiotic-resistant bacteria and endotoxin associated with the land application of Biosolids. Canadian Journal of Microbiology 2007;53:616 -622.
5. Buelow E, Bayjanov JR, Willems RJ, Bonten MJ, Schmitt H, Van Schaik W. The microbiome and resistome of hospital sewage during passage through the community sewer system, bio Rxiv 2017, 216242.
6. Chowdhury S. Exposure assessment for trihalomethanes in municipal drinking water and risk reduction strategy. Science of the Total Environment 2013;463:922-930.
7. Cruickhank R, Dugid JP, Marmoin BP, Swain RHA. Medical Microbiology: The practice of Medical Microbiology. 12th Edn., Churchil Livingstone, Edinburg, London 1975.
8. Edwards R, Ewing WN. Identification of enterobacteriaceae. 3rd Edn., Burgess Publishing Co., Minnesota 1972
9. Elmanama AA, ElKichaoui AY, Mohsin M. Contribution of hospital wastewater to the spread of antibiotic resistance in comparison to non-health institution. Al-Aqsa University Journal (Natural Sciences Series)

2006;*10*:108-121.
10. Fridrich B, Kromar D, Dalmacija B, Molnar J, Peši´c V, Kragulj M *et al*. Impact of wastewater from pig farm lagoons on the quality of local groundwater. Agricultural Water Management 2014;135:40-53.
11. Gautam AK, Kumar S, Sabumon PC. Preliminary study of physicochemical treatment options for hospital wastewater. Journal of Environmental Management 2007;83:298-306.
12. Ibrahim C, Hassen A, Pothier P, Mejri S, Hammami S. Molecular detection and genotypic characteri- zation of enteric adenoviruses in a hospital wastewater. Environmental Science and Pollution Research International 2018;25(11):10977-10987.
13. Jolibois B, Guerber M, Vassal S. Glutaraldehyde in hospital wastewater. Archives of Environmental Contamination Toxicology 2002; 42:137-144.
14. Jolibois B, Guerbet M. Hospital wastewater genotoxicity. Annals of Occupational Hygiene 2006;50(2):189-196.
15. Oyeleke SB, Istifanus N, Manga SB. The effects of hospital solid waste on the receiving environment. International Journal of Integrative Biology 2008;3(3):191-195.
16. Pradhan B, Mishra SK. Multiple drug resistance in bacterial isolates from liquid wastes generated in central hospitals of Nepal. Kathmandu University Medical Journal 2010;8(1):40-44.
17. Pruden A, Pei R, Storteboom H, Carlson KH. Antibiotic resistance genes as emerging contaminants: studies in Northern Colorado. Environmental Science and Technology 2006;40:7445-7450.
18. Reinthaler F, Posch F, Feierl G, Wust D, Haas G, Ruckenbaur F *et al*. Antibiotic resistance of *E. coli* in sewage and sludge. Water Research 2003;37:1685-1690.
19. Resende ACB, Ascenso Soares R, Santos DB, Montalvao ER and Carmo Filho JR. Detection of antimicrobial resistant gram-negative bacteria in hospital effluents and in the sewage treatment station of Goiania, Brazil 2009; 33(4):385-391.
20. Sasakova N, Veselitz-Lakticova K, Hromada R, Chvojka D, Kosco J, Ondrasovic M. Contamination of Individual sources of drinking water located in environmentally polluted central spis region (Slovakia). Journal of Microbiology, Biotechnology and Food Science 2013;3:262-265.
21. WHO. Enterohaemorrhagic Escherichia coli (EHEC) Fact sheet N◦125 2011. Available online at: http://www.who.int/mediacentre/factsheets/fs125/en/