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Antimicrobial Resistance Pattern against *E. coli* and *Salmonella spp.* in Environmental Effluents

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ABSTRACT

Hospitals (medical & veterinary) and slaughterhouse effluents were the most contaminating effluents and need to be paid more attention due to pathogenic bacteria related to animal and public health concern. Two bacterial isolates such as *E. coli* and *Salmonella* from six medical hospitals, five veterinary hospitals and five slaughter houses were isolated to find out the antibiotic resistance pattern by using disc diffusion method. The antibiotic resistance patterns of identified isolates showed that Ampicillin, Ciprofloxacin, Enrofloxacin, Pefloxacin, Colistin, Erythromycin, Oxytetracycline were 100%, Doxycycline was 83%, Gentamycin was 50% and Neomycin was 33% resistance to medical isolates and Ampicillin, Enrofloxacin, Pefloxacin and Erythromycin were 100%, Ciprofloxacin was 40%, Colistin was 60%, Doxycycline was 80%, Gentamycin was 20%; Neomycin and Oxytetracycline 80% resistance to veterinary hospital isolates and Ampicillin, Enrofloxacin, Ciprofloxacin, Pefloxacin, Colistin, Oxytetracycline, Gentamycin, Doxycycline and Erythromycin were 100% and Neomycin was 40% resistance to slaughter houses isolates of *E. coli*. The level of resistance of *Salmonella* positive isolates was found Ampicillin, Enrofloxacin, Pefloxacin, Gentamycin and Erythromycin to 100%, Ciprofloxacin was 67%, Oxytetracycline was 33% but Colistin and Neomycin was found sensitive to the isolates from both medical and veterinary hospital. Results indicated that hospitals and slaughter houses waste effluent has multiple-antibiotic resistance against *E. coli* and *Salmonella.*

Key words: Antimicrobial resistance, effluents, hospital, slaughterhouse, *E. coli*, *Salmonella*.

INTRODUCTION

The emergence of bacteria resistant to antibiotics is common in areas where antimicrobial treatments are used. Antibiotics are used extensively to prevent or to treat microbial infections in human and veterinary medicine and residues of antibiotics persist in the products of food animals [1] which causing important public health hazard. Apart from their use in aquaculture, they are also employed to promote more rapid growth of livestock [2]. Heavy use of antibiotics for medical and veterinary purposes as well as the domestic and agricultural use of pesticides and related compounds [3] caused significant antibiotic contamination of the natural environment and consequent development of resistance in communities [4]. One of the ways multi-resistant bacteria may be introduced into the biocoenosis and into humans via environment [5]. The micro flora of hospital wastewaters is composed by saprophytic bacteria from the atmosphere, soil, medical devices and water employed in the hospital practice; the pathogens are mainly released with the patient excreta [6]. Bacteria have developed different mechanisms to render ineffective the antibiotics used against them. The genes encoding these defense mechanisms are located on the bacterial chromosome or on extra chromosomal plasmids, and are transmitted to the next generation. The use of urban wastewater in agricultural fields is a centuries old practice [7]. In countries, where treatment and safe effluent disposal facilities are limited, sewage is used to irrigate fodders, ornamental and food crops including vegetables [8]. Wastewater treatment allows waters to be reused for irrigation in agriculture or released directly in aquatic environments. The presence of antibiotic-resistant bacteria in effluents [9] as well as high levels of antibiotic compounds in wastewater treatment plants has been addressed in several studies, creating a growing concern about their impact on animal and human health [10]. Water-borne bacterial pathogens such as *E. coli* 0157, *Salmonella spp*., *Shigella spp*. and *Vibrio cholerae* can lead to diarrhoeal outbreaks that may have serious medical and economic (livestock) implications [11]. Antimicrobial-resistant infections add 6.4-12.7 hospital days per patient and $26 billion to $35 billion total in healthcare costs [12]. In majority cases, effluent is discharged directly in water bodies in low-lying areas, natural khals and rivers with storm water for natural degradation without any treatment. Presence of multidrug resistant *E. coli* and *Salmonella* in drinking water can act as a vehicle to disseminate antibiotic resistance to other bacteria. On the other hand, arthropod vectors may transmit the resistance bacteria from drain to open food un-hygienically prepared besides the roads, rivers or other natural water source areas. Considering all the above facts, the present study was undertaken to investigate the scenario of antimicrobial resistance pattern against *E. coli* and *Salmonella* in environmental samples.

MATERIALS AND METHODS

Description of study area

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Chittagong is the second largest city, located in southeastern part of Bangladesh. Its estimated population stands at over 5 million and population density per square km is 15276 (http://www.dmb.gov.bd). To provide health care services to her metropolitan civilian, livestock and poultry, it has a number of medical and veterinary hospitals including General hospital, Upazila health complex, Family welfare center, TB hospital, Infectious disease hospital, Diabetic hospital, Mother and children hospital and Police hospital. From which six medical hospitals, five veterinary hospitals and five slaughterhouses were selected randomly.

Study duration and sample collection

The study was conducted during the period of September to December, 2012. Samples were collected from final effluents of medical hospitals, veterinary hospitals and slaughterhouses. About 250 ml pre-sterilized glass bottles were used to transport the samples to PRTC (Poultry Research and Training Centre) laboratory for analysis.

Media used

Peptone water (Oxoid Ltd., P^H: 6.2±0.0) was used as primary enrichment media for *E. coli* and *Salmonella.* Five selective media were used for the isolation of the bacteria. The MacConkey agar (Oxoid Ltd., P^H 7.4±0.2) and EMB agar (Merck, P^H: 7.1±0.2) were used for *E. coli*, XLD agar (Oxoid Ltd., P^H 7.4±0.2), BGA agar (Merck, P^H: 6.9±0.2) and TSI agar (Oxoid Ltd., P^H: 7.2±0.2) for *Salmonella*. Muller Hinton agar (Biotec, P^H: 7.3±0.1) was used for determination of antibiotic resistant bacteria.

Culture protocol for isolation and identification *E. coli*

For the isolation of *E. coli*, 1ml of water sample was inoculated in screw cap test tube containing buffer peptone water (primary enrichment media) and incubated overnight at 37^0C. After primary enrichment culture of the buffer peptone water containing bacteria was streaked on MacConkey agar and incubated for another 24 hours at 37^0C. After overnight incubation the bacterial growth was observed. The pink color colony suspected for *E. coli.* Then again sub-culture was done on EMB agar and incubated as the above mentioned time period. The growth of characteristic metallic sheen like colony was confirmed to *E. coli* positive. It was further confirmed by Gram's staining and Indole biochemical test.

Table1: Isolation and identification *E. coli* in culture on MacConkey, EMB Agar and Indole test, TSI stab and Gram staining

Sample	MacConkey	EMB	Indole test	TSI Stab **(slant/butt)**	Microscopic features
MH N=6	6 (+VE)	6 (+VE)	6 (+VE)	4 (A/A,G) 2(A/A)	Gram-negative, rod
VH N=5	5 (+VE)	5 (+VE)	5 (+VE)	4 (A/A,G) 1 (A/A)	Gram-negative, rod
SH N=5	5 (+VE)	5 (+VE)	5 (+VE)	5(A/A,G)	Gram-negative, rod

MH= Medical Hospital; VH= Veterinary Hospital; SH= Slaughterhouse; +VE = Positive; A/A=Yellow/Yellow; A/A, G=Yellow/Yellow with gas bubbles

Table-2: Isolation and identification of *Salmonella spp.* on XLD, BGA agar and TSI stab and Gram staining

Sample	XLD	BGA	TSI Stab (slant/butt)	Microscopic features
MH	4 (+VE)	4 (+VE)	3 (K/A,G,H_2S)	4 (Gram-negative, pink colored, small rod)
N=6	2 (-VE)	2 (-VE)	1 (K/A)	2 (ND)
			2 (ND)	
VH	3 (+VE)	3 (+VE)	3 (K/A,G,H_2S)	3 (Gram-negative, pink colored, small rod)
N=5	2 (-VE)	2 (-VE)	2 (ND)	2 (ND)
SH	5 (-VE)	5 (-VE)	5 (ND)	5 (ND)
N=5				

MH= Medical Hospital; VH= Veterinary Hospital; SH= Slaughterhouse; +VE= Positive -VE= Negative; K/A= Red/Yellow; K/A, G, H_2S = Red/Yellow with gas bubbles and black precipitate; ND = Not detected

Salmonella

For the isolation of *Salmonella*, 1ml of water sample was inoculated in screw cap test tube containing buffer peptone water (primary enrichment media) and incubated for 24 hours at 37^0C. After primary enrichment sample from buffer peptone was picked up and streaked on both XLD and BGA agar. The agar plates then were incubated at 37^0C for 24 hours. After development of characteristic colony the positives were selected for biochemical test (TSI stab) to confirm *Salmonella.*

Gram's staining

Gram's staining was performed as per procedures described by [13] to determine the size, shape and arrangement of bacteria. Therefore**,** the suspected colonies were taken over a slid to make a thin smear that was done by sliding the edge of another glass slide across the glass slide containing the sample and then allowed it to air dry. The smear was then heat fixed by quickly passing it two to three times through a flame. After fixation the Gram's staining was done as follows: Crystal violet was used for two minutes, Gram's iodine for 1 minute, Acetone for 5-7 seconds and finally, Safranin for 1 minute. Rinsing was done gently with tape water after every step. The slide was then observed by microscope under 100X with immersion oil and characterization of bacteria was done.

Table-3: CS-test for isolates of *E. coli*

Sample	Antibiotic disc used									
	AMP	CIP	CL	DO	E	ENR	GEN	N	PF	TA
MH	6 (R)	6 (R)	6 (R)	5 (R)	6 (R)	6 (R)	3 (R)	2 (R)	6 (R)	6 (R)
N=6				1 (I)			3 (S)	2 (S)		
								2 (I)		
VH	5 (R)	3 (R)	2 (R)	4 (R)	5 (R)	5 (R)	1 (R)	4 (R)	5 (R)	4 (R)
N=5		2 (S)	3 (S)	1 (I)			2 (S)	1 (S)		1 (I)
							2 (I)			
SH	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)	5 (R)	2 (R)	5 (R)	5 (R)
N=5								3 (I)		

MH= Medical Hospital; VH= Veterinary Hospital; SH= Slaughterhouse; AMP=Ampicillin; CIP= Ciprofloxacin; CL= Colistin; DO= Doxycycline; E= Erythromycin; ENR= Enrofloxacin; GEN= Gentamycin; N= Neomycin; PF= Pefloxacin; TA= Oxytetracycline; R= Resistance; I= Intermediate; S= Sensitive

Table-4: Prevalence of antibiotic resistance pattern against *E. coli* positive isolates

Antibiotic	Pattern	Medical Hospital	Veterinary Hospital	Slaughterhouse
AMP	Resistance	6 (100%)	5 (100%)	5 (100%)
CIP	Resistance	6 (100%)	2 (40%)	5 (100%)
	Sensitive	0 (0%)	3 (60%)	0 (0%)
CL	Resistance	6 (100%)	2 (40%)	5 (100%)
	Sensitive	0 (0%)	3 (60%)	0 (0%)
DO	Resistance	5 (83%)	4 (80%)	5 (100%)
	Intermediate	1 (17%)	1 (20%)	0 (0%)
E	Resistance	6 (100%)	5 (100%)	5 (100%)
ENR	Resistance	6 (100%)	5 (100%)	5 (100%)
GEN	Resistance	3 (50%)	1 (20%)	5 (100%)
	Intermediate	0 (0%)	2 (40%)	0 (0%)
	Sensitive	3 (50%)	2 (40%)	0 (0%)
N	Resistance	2 (33%)	4 (80%)	2 (40%)
	Intermediate	2 (33%)	0 (0%)	3 (60%)
	Sensitive	2 (33%)	1 (20%)	0 (0%)
PF	Resistance	6 (100%)	5 (100%)	5 (100%)
TA	Resistance	6 (100%)	4 (80%)	5 (100%)
	Intermediate	0 (0%)	1 (20%)	0 (0%)

AMP=Ampicillin; CIP= Ciprofloxacin; CL= Colistin; DO= Doxycycline; E= Erythromycin; ENR= Enrofloxacin; GEN= Gentamycin; N= Neomycin; PF= Pefloxacin; TA= Oxytetracycline

Biochemical test

a. Indole test for *E. coli*

The tube of tryptone broth was inoculated with a small amount of pure culture at 37^0C for overnight. A positive Indole test is indicated by the formation of a pink to red color ("cherry-red ring") in the reagent layer on top of the medium within seconds of adding the reagent.

b. TSI slant for *Salmonella* and *E. coli*

A straight inoculating needle was used to pick up isolated colony from culture of isolates. The TSI slant was inoculated by stabbing the butt down to the bottom, and then streaked over the surface of the slant. The TSI slant was then incubated overnight at temperature of 37^0C. The positive result for *Salmonella* and *E. coli* were detected based on the properties.

Cultural Sensitivity (CS) Test at Muller Hinton Agar

After confirmation of isolates as *E. coli* and *Salmonella,* antimicrobial susceptibility of the isolates was determined by using the micro disc diffusion method, and the method was used according to guidelines established by *Clinical and Laboratory Standards Institute [18]*. Antibiotics selected for susceptibility testing included a panel of

antimicrobial agents of interest to the poultry industry and public health authorities. From the range of antimicrobial drugs, 10 were selected on the basis of their range of activity against entero-bacteria on their use in local poultry farming and human medicine. Veterinary antibiotics were chosen due to their use as therapeutic, prophylactic or growth promoting agents in livestock industry and human antibiotics were selected on the basis of their use and /or importance in human medicine. The following antibiotics and disc potencies were used for *E. coli* and *Salmonella*: GEN: Gentamicin (10mcg), DO: Doxycycline (30mcg), CIP: Ciprofloxacin (5mcg), TA: Oxytetracycline (30mcg), ENR: Enrofloxacin (5mcg), AMP: Ampicillin (25mcg), CL: Colistin (10mcg), N: Neomycin (30mcg), E: Erythromycin (15mcg) and PF: Pefloxacin (5mcg). Measurement of the growth inhibition zone permitted the classification of each isolates as susceptible, intermediate and resistant according to data provided by HiMedia Laboratories pvt. Limited, Mumbai.

Table-5: CS-test for *Salmonella* positive isolates

Sample	Antibiotic disc used									
	AMP	CIP	CL	DO	E	ENR	GEN	N	PF	TA
MH N= 4	4 (R)	4 (R)	4 (R)	4 (I)	4 (R)	4 (R)	4 (R)	4 (S)	4 (R)	4 (I)
VH N=3	3 (R)	3 (S)	3 (S)	3 (S)	3 (R)	3 (R)	3 (R)	3 (I)	3 (R)	3 (R)

MH= Medical Hospital; VH= Veterinary Hospital; SH= Slaughterhouse; AMP= Ampicillin; CIP= Ciprofloxacin; CL= Colistin; DO= Doxycycline; E= Erythromycin; ENR= Enrofloxacin; GEN= Gentamycin; N= Neomycin; PF= Pefloxacin; TA= Oxytetracycline; R= Resistance; I= Intermediate; S= Sensitive

Data analysis

Data obtained was imported to the Microsoft Office Excel-2007 and transferred to the software STATA/IC-11 for analysis. Descriptive statistics was done by using the STATA software and expressed as percentages of different variables like resistance, intermediate and sensitivity pattern of antimicrobials.

RESULTS

In table 1, culture of effluent on MacConkey agar for the isolation of *E. coli* were able to produce bright pink colonies (non-mucoid) due to fermentation of lactose, while lactose negative organisms (*Salmonella, Shigella*) have only peptone as energy sources were colorless. Similarly, sub cultured on EMB agar showed very dark colonies and almost black colonies when observed directly against the light. By reflected light, a green sheen were seen which is due to the precipitation of methylene blue in the medium and the very high amount of acid produced from lactose fermentation are the characteristics to *E. coli.* All samples were found tests positive (+) in the presence of Indole indicated by the red reagent layer after addition of Kovács reagent. In TSI stabbing, suspected *E. coli* of the 13 samples were shown yellow slant and yellow butt with gas production and 3 samples were shown yellow slant and yellow butt without any bubble formation. In Gram's staining, positive colonies able to revealed Gram-negative, rod shaped bacteria under microscope.

In table 2, colonies were isolated as positive (+) on the basis of characteristic colony color and morphology cultured on XLD and BGA agar. Positive isolates were found in 4 Medical hospital samples and 3 Veterinary hospital samples. On BGA, *Salmonella* colonies were surrounded by a pink zone, whereas on XLD agar, the colonies appeared as black centered because of H_2S production. Non-*Salmonella* colonies appeared white with yellow background on XLD plates, and on BGA plate's colonies were white. In case of TSI stab suspected *Salmonella,* the 1 sample showed red slant and yellow butt and 6 samples were shown red slant yellow butt with bubbles (gas) and black precipitation that was confirmatory to *Salmonella.* Gram-negative, pink colored small rod shaped bacteria were found under microscope in Gram staining. Based on the characteristic growth and colony color, it assumed that organisms are *Salmonella spp.*

In table 3, out of 16 samples *E. coli* was found positive in all the Medical hospital, Veterinary hospital and Slaughterhouse samples. Resistance to tested antibiotics was found variable among them. AMP, E, ENR and PF were resistance in all isolates. TA, DO, CIP, CL, GEN and N were found resistant to 15, 14, 14, 13, 9 and 8 isolates, respectively. On the other hand, GEN, N, CL and CIP were found sensitive to 5, 3, 3 and 2 isolates, respectively. But, DO, N, GEN and TA were intermediate sensitive to some isolates.

In table 4, the prevalence of resistance exhibited by isolates of *E. coli* to AMP, CIP, CL, E, ENR, PF and TA were 100% followed by DO (83%), GEN (50%) and N (33%) for Medical hospital effluents. On the other hand, sensitivity to GEN and N was 50% and 33%, respectively. Resistance to veterinary hospitals isolates of *E. coli* showed 100% to AMP, E, ENR and PF followed by 80% to DO, N and TA, 40% to CL and CIP, 20% to GEN. Besides, sensitivity was found 40% and 20% to GEN and N, respectively. The isolates of *E. coli* from Slaughterhouse effluents were shown 100% resistance to AMP, CIP, CL, DO, E, ENR, GEN, PF and TA and 40% to N.

In table 5, out of 16 samples *Salmonella* was found positive in 4 Medical hospital and 3 Veterinary

hospital samples. AMP, E, ENR, GEN and PF were resistant in all isolates. CL was found sensitive for all sample isolates. DO and TA were intermediate sensitive to Medical hospital isolates but was resistance to Veterinary hospital samples. On the other hand, CIP was found sensitive to Veterinary hospital and resistance to Medical hospital isolates.

In table 6, the prevalence of *Salmonella* positive isolates were found 100% resistance to AMP, CIP, E, ENR, GEN and PF but 100% sensitive to CL, N and other antibiotics were intermediate sensitive in Medical hospital samples. On the other hand, 100% resistances were found to AMP, E, ENR, GEN and PF but 100% sensitive to CIP, CL, DO and other antibiotics were intermediate sensitive in Veterinary hospital effluents.

Tabe-6: Prevalence of antibiotic resistance pattern against *Salmonella* positive isolates

Antibiotic	Pattern	Medical Hospital	Veterinary Hospital
AMP	Resistance	4 (100%)	3 (100%)
CIP	Resistance	4 (100%)	0 (0%)
	Sensitive	0 (0%)	3 (100%)
CL	Sensitive	4 (100%)	3 (100%)
DO	Intermediate	4 (100%)	0 (0%)
	Sensitive	0 (0%)	3 (100%)
E	Resistance	4 (100%)	3 (100%)
ENR	Resistance	4 (100%)	3 (100%)
GEN	Resistance	4 (100%)	3 (100%)
N	Intermediate	0 (0%)	3 (100%)
	Sensitive	4 (100%)	0 (0%)
PF	Resistance	4 (100%)	3 (100%)
	Intermediate	0 (0%)	0 (0%)
TA	Resistance	0 (0%)	3 (100%)
	Intermediate	4 (100%)	0 (0%)

AMP= Ampicillin; CIP= Ciprofloxacin; CL= Colistin; DO= Doxycycline; E= Erythromycin; ENR= Enrofloxacin; GEN= Gentamycin; N= Neomycin; PF= Pefloxacin; TA= Oxytetracycline

DISCUSSION

In recent years antimicrobial resistance in bacteria of animal origin and its impact on human health have drawn much attention worldwide. Tetracycline resistance was the most common type of resistance observed and the most prevalent resistance in *E. coli* from all isolates but relatively lower resistance was observed for *Salmonella*. This finding is not surprising because tetracycline has been widely used in therapy and to promote feed efficiency in animal production systems since its approval in 1948 [14]. Persistence of tetracycline resistance was reported in animal coliform a decade after it was no longer used in feed or for treatment. Earlier research [15] found that methicillin-resistant *Staphylococcal* hospital isolates was 57.1% resistance to tetracycline. On the other hand, multidrug-resistance in *Salmonella typhimurium* isolated from swine shown 90% resistance to tetracycline [16]. Several researchers [17] found 90.5% resistance of downstream water and upstream water isolates to tetracycline. Both findings were agreed with our present research. *E. coli* isolates from water sample of Cypress channel found 74.4% resistant to tetracycline [18]. Generally, amoxicillin is used to treat many different types of infections caused by bacteria, such as ear infections, bladder infections, pneumonia, gonorrhea, and *E. coli* or *salmonella* infection [19]. Amoxicillin resistance was very common among the isolates from all study areas. A research [20] conducted with the isolation of 79 *Salmonella* strains from river and lake waters from northern Greece which were susceptible to amoxicillin. On the other hand, research showed that resistance develops 45% to amoxicillin-clavulanic acid and ampicillin for *Salmonella* [21] and found 21.5% resistance for *E. coli* [22]. These findings showed less development of resistance than our present findings. Resistance pattern for the isolates of *E. coli* from poultry farm fecal waste was 90% resistance to amoxicillin [23] which was similar to our findings. *Staphylococcus* resistance to Oxacillin, Penicillin and Ampicillin was 100% and Cephalothin was 92.4% [24] those were agreed to present findings. Some time it appears to be completely eliminated unchanged in the urine that may contribute in development of resistance in environment [25]. In the present study resistance to Gentamicin was mainly found against *E. coli* but *Salmonella* was not exhibited such resistance as *E. coli*. Similarly, both resistance and susceptibility was found in *E. coli* strains against gentamicin in a research finding [26].

In veterinary practice, fluoroquinolones was also very extensively used for both therapeutic and non-therapeutic purposes. In the study the level of resistance is higher in medical hospital rather than veterinary hospitals isolates. This might be due to relatively newer introduction of ciprofloxacin and recent introduction of Pefloxacin in animal health division of Bangladesh. Fluoroquinolones resistance has increased significantly over the past decade in the United States, exceeding 25% resistance in outpatient *E. coli* samples in some areas. The resistance rate to either ciprofloxacin or to

levofloxacin increased from 2.8% (1998-2003) to 11.8% (2004-2007) in clinical isolates in Taiwan and about 25% of healthy individuals living in Barcelona [27]. A study [28] was conducted a study by twenty-one patients with multidrug-resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa* pneumonia were treated with nebulized colistin. Based on the antibiotic-resistance patterns, previous study [29] observed that all isolates tested were resistant to tetracycline (5%-95%), ampicillin (10%-80%), chloramphenicol (5%-80%) and erythromycin (50%-100%). In recent years, testing of *Salmonella* isolates from different environments has shown an increasing proportion of multidrug resistant *Salmonella spp.* According to the information [30] about antimicrobial resistance among *Salmonella* strains isolated from environmental sources and food showed a differentiated incidence rate of resistant strains among isolates obtained from developed and developing countries. In India, 82% of the strains isolated from seafood products presented antimicrobial resistance [31], whereas in Vietnam, antimicrobial resistance was observed in 11.1% of strains [32]. In this study *Salmonella* showed no resistance against colistin and neomycin but surprisingly shown multidrug resistance against other tested antibiotics, similar to the findings of Molla *et al.*, [21, 33]. Moreover, bacteria are able to horizontally acquire resistance via uptake of foreign DNA by means of conjugation, transduction or transformation [34]. In this context, mobile genetic elements such as plasmids, transposable elements or integron-specific gene cassettes play an important role [35], these elements mainly encode enzymes for modification or inactivation of antibiotics, efflux systems, or enzymes catalyzing target-site modifications [33]. In the present study, the slaughter house isolates *E. coli* shown more resistance than hospital isolates this is might be due to aggregation of clinically infected and carrier animal in slaughter house alone with opening and drainage of carcass after slaughtering and chance to contaminating the environment.

CONCLUSION

Two bacterial isolates such as *E. coli* and *Salmonella* from medical hospitals, veterinary hospitals and slaughterhouses were isolated to find out the antimicrobial resistance pattern by using disc diffusion method. Resistance pattern of *E. coli* were more in slaughterhouse isolates in comparison to hospitals. The prevalence of *Salmonella* positive isolates were found in only three isolates. Overall results indicated that hospitals and slaughterhouses' waste effluents have multiple-antibiotic resistance among *E. coli* and *Salmonella*. For this purpose, it is important to make a more detailed assessment of the significance of culture-dependent and laboratory-based methods in relation to conditions found in the environment.

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CONFLICTS OF INTEREST

The authors declare that they have no competing interests.

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