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Research Article

Multi-Drug Resistance Pattern of *Escherichia coli* Isolated from Hospital Effluent and Determination of Tetracycline Resistance Gene

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Abstract | The Current study was conducted to investigate anti-microbial resistance pattern of *Escherichia coli* (*E. coli*) isolated from hospital effluent and the occurrence of tetracycline resistance determinant genes: *tet (A), tet (B)* and *tet (C)* in tetracycline-resistant isolates. Effluent samples were collected from 15 randomly selected hospitals of Chittagong Metropolitan area for the isolation of *E. coli* based on cultural and biochemical properties. The isolated *E. coli* were screened for the anti-microbial susceptibility against 10 commonly used anti-microbials in the hospitals. Tetracycline-resistant isolates (13 out of 15) were employed for polymerase chain reaction (PCR) to determine the presence of tetracycline resistance determinant genes. *E. coli* isolates were resistant (100%) against amoxicillin and cephalothin followed by tetracycline (86.67%), sulphamethoxazole-trimethoprim (80%), ceftriaxone (73.33%), nalidixic acid (66.67%), enrofloxacin (66.67%) and chloramphenicol (20%). Isolates were sensitive against gentamycin (86.67%) followed by neomycin (73.33%), chloramphenicol (66.67%), ceftriaxone (20%) and sulphamethoxazole-trimethoprim (20%). Moreover, all isolates showed multi-drug resistance pattern. The prevalence of tetracycline resistance determinants were 53.85%, 15.38% and 0% for *tet* (A), *tet* (B) and *tet* (C) genes respectively. Both *tet* (A) and *tet* (B) genes were positive in 15.38% isolates. None of the isolates possessed all three genes or *tet* (B) and *tet* (C) genes or *tet* (A) and *tet* (C) genes collectively. A 53.85% isolates possessed one or more of the tested genes whereas 46.15% isolates had no tested genes. The study revealed that hospital effluent might be one of the major sources of the multi-drug resistant *E. coli* in environment and, to overcome this problem, the hospital effluent should be treated efficiently.

Keywords | Anti-microbial resistance, *E. coli*, Hospital effluent, *tet*, PCR

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INTRODUCTION

Now-a-days antibiotic resistance has become one of the world's most pressing public health issues. The increasing trend of antibiotic resistance has repeatedly been placed on the global agenda as a threat to functioning health systems (World Health Assembly, 2005). CDC (Center for Disease Control and Prevention, USA) has reported that at least 2 million people become infected by anti-microbial resistant bacteria and 23,000 people die in the United States every year (CDC, 2013). Primary sources of antibiotic contamination of the environment are waste water (here termed as effluent) from pharmaceutical plants, disposed unused antibiotics and excreta of humans and animals treated with antibiotics (Kümmerer, 2009). According to Martins et al. (2008), hospital effluent is an important contributory source of antibiotics to the environment. Chagas et al. (2011) state that hospital sewage receives antibiotics, antibiotic-metabolites and resistant bacteria through urine and feces. Again hospi-

tal provides an environment for the MDR (Multi-drug resistant) bacteria, making the treatment options limited and expensive. Indiscriminate use of antibiotics in human treatment, veterinary purposes and agriculture causes significant antibiotic contamination of the natural environment. Again, one of the major sources for the development of antibiotic resistance in organism is the presence of antibiotics in the environment (Kümmerer, 2009).

Diwan et al. (2009) found that, in India, hospital effluent contains different types of drug residue ranging 1.4 to 236.6 µg/ml. There is a positive correlation between antibiotic prescription and their residue level in hospital effluent (Diwan et al., 2009). Due to strategic similarity, same impact can be expected in hospital effluent of Bangladesh. *Escherichia coli* is one of the common microbial floras of gastrointestinal tract of human and animals. They are considered as an indicator of fecal contamination of food. *E. coli* produces different diseases in human, animals and poultry (Daini et al., 2004). Again, *E. coli* is responsible for gastroenteritis, cystitis, pneumonia and septicemia (mostly nosocominal origin) in non-hospitalized patients. In both human and veterinary medicine, tetracycline, one of the broad spectrum antibiotics, is commonly used for treating *E. coli* infection. It is also used for growth promotion and prophylaxis in poultry industry. Now a days *E. coli* shows resistance to different drugs.

According to Chopra and Roberts (2001) in *E. coli*, the main tetracycline resistance mechanism is the efflux of drug from inside to outside of the cell through specific tetracycline operating pump (*tet*) (Chopra and Roberts, 2001). There are five genes encoding for energy-dependant efflux proteins. They are *tet*(A), *tet*(B), *tet*(C), *tet*(D) and *tet*(E) (Roberts, 2005). In *E. coli*, *tet*(A) and *tet*(B) genes are more prevalent than others (Tuckman et al., 2007). According to Wilkerson et al. (2004) 60% of the tetracycline-resistant *E. Coli* O157:H7 carries *tet*(B) gene. However, there is limited information regarding antibiotic resistance pattern of *E. coli* isolated from hospital effluent in Bangladesh. Therefore, the present study was conducted to investigate the anti-microbial resistance pattern of *E. coli* isolated from hospital effluent and to determine the resistance genes in tetracycline-resistant isolates.

MATERIALS AND METHODS

The study was conducted during the period of March to May, 2015 in Chittagong Metropolitan area, Bangladesh. About 15 randomly selected hospitals, having at least 200 beds, were included in this study. A total of 15 effluent samples (1 from each hospital) were collected in sterilized falcon tubes and were immediately send to PRTC (Poultry Research and Training center) in Chittagong Veterinary and Animal Sciences University (CVASU) for details microbiological investigation. For primary enrichment, samples were inoculated in buffer peptone water (BPW) (Oxoid Ltd, P^H:6.2±0.0) and incubated at 37°C for overnight. After primary enrichment, *E. coli* were isolated using MacConkey agar (Oxoid Ltd, P^H: 7.4±0.2), EMB agar (Merck, P^H: 7.1±0.2).

On the basis of colonial morphology, positive isolates were finalized for biochemical test (Indole test using Kovác's reagent) and Gram's stain property testing. Then isolated *E. coli* were screened for anti-microbial sensitivity by using standard Kirby Bauer disc diffusion method following the guidelines of Clinical and Laboratory Standard Institute (CLSI, 2011). About 10 (from 6 different groups) anti-microbial agents (Table 1) of public health interest were selected for cultural sensitivity (CS) testing. *E. coli* isolates showing resistance against at least three groups of anti-microbial agents (≥3) were defined as multi-drug resistant (MDR) isolates (Li et al., 2014).

Table 1: Concentrations and diffusion zone breakpoints for anti-microbials used (CLSI, 2011)

Group of anti-microbials	Anti-microbial agent (code)	Disc content*	Diffusion zone breakpoint (diameter in mm)		
			R	I	S
β-lactam antibiotics	Amoxicillin (AML)	10µg	≤13	14-17	≥18
	Cephalothin (KF)	30µg	≤14	15-17	≥18
	Ceftriaxone (CRO)	30µg	≤19	20-22	≥23
Tetracyclines	Tetracycline (TE)	30µg	≤14	15-18	≥19
Aminoglycosides	Gentamycin (CN)	10µg	≤12	13-14	≥15
	Neomycin (N)	30µg	≤12	13-16	≥17
Phenicols	Chloramphenicol (C)	30µg	≤12	13-17	≥18
Quinolones	Nalidixic acid (NA)	30µg	≤13	14-18	≥19
	Enrofloxacin (ENR)	5µg	≤17	18-21	≥22
Sulfonamides	Sulphamethoxazole-trimethoprim (SXT)	23.75µg+ 1.25µg	≤10	11-15	≥16

***Manufacturer of disc:** Oxoid Limited, Basingstoke, Hampshire, England; **S:** Sensitive; **I:** Intermediate; **R:** Resistant

The tetracycline resistant isolates were subjected to conventional PCR using following primers: a) For *tet*(A) gene F: 5′-GGCGGTCTTCTTCATCATGC-3′ and R: 5′-CGGC AGGCAGAGCAAGTAGA-3′, b) For *tet*(B) gene F: 5′-CATTAATAGGCGCATCGCTG 3′ and R: 5′-TGAAGGTCATCGATAGCAGG-3′, c) For *tet*(C) F: 5′-GCTGTAGGCATAGGCTTGGT -3′ and R: 5V-GCCGGAAGCGAGAAGAATCA-3′ (Boerlin et al., 2005). 1.5 % agarose gel (W/V) was used to visualize the PCR product. All data were analyzed using the software STATA/IC-11.

Table 2: Antimicrobial resistance pattern of *E. coli* isolates

Antibiotics	Total isolates	Anti-microbial sensitivity pattern		
		Sensitive % (n)	Intermediate %(n)	Resistant %(n)
TE	15	13.33(2)	0(0)	86.67(13)
CN	15	86.67(13)	6.67(1)	6.67(1)
AML	15	0(0)	0(0)	100(15)
CRO	15	20(3)	6.67(1)	73.33(11)
C	15	66.67(10)	13.33(2)	20(3)
SXT	15	20(3)	0(0)	80(12)
KF	15	0(0)	0(0)	100(15)
N	15	73.33(11)	13.33(2)	13.33(2)
NA	15	13.33(2)	20(3)	66.67(10)
ENR	15	13.33(2)	20(3)	66.67(10)

n: Number; **TE:** Tetracycline; **CN:** Gentamycin; **AML:** Amoxicillin; **CRO:** Ceftriaxone; **C:** Chloramphenicol; **SXT:** Sulphamethoxazole- trimethoprim; **KF:** Cephalothin; **N:** Neomycin; **NA:** Nalidixic acid; **ENR:** Enrofloxacin

Table 3: Multi-drug resistance pattern of *E. coli* isolates

Sample No.	Resistant against anti-microbial agents	No of groups
01	TE, AML, CRO, SXT, KF, NA, ENR	4
02	TE, AML, CRO, SXT, KF, NA, ENR	4
03	AML, CRO, KF, C, N	3
04	TE, AML, CRO, SXT, KF, NA, ENR	4
05	AML, CRO, C, KF, NA,	3
06	TE, AML, CRO, KF, N	3
07	TE, AML, CRO, CN, SXT, KF, NA, ENR	5
08	TE, AML, CRO, SXT, KF, NA, ENR	4
09	TE, AML, SXT, KF, NA, ENR	4
10	TE, AML, SXT, C, KF, NA, ENR	5
11	TE, AML, CRO, SXT, KF, ENR	4
12	TE, AML, CRO, SXT, KF	3
13	TE, AML, SXT, KF, NA	4
14	TE, AML, SXT, KF, ENR	4
15	TE, AML, CRO, SXT, KF, NA, ENR	4

For abbreviation see Table 2

RESULTS

On the basis of colonial morphology, biochemical and staining properties, all the samples (100%) were positive for *E. coli*. But anti-microbial resistance pattern of *E. coli* isolates were highly diversified. Among 15 isolates, all (100%) isolates showed resistance against amoxicillin and cephalothin and 86.67% isolates were resistant to tetracycline. Highest level of sensitivity was found against aminoglycoside group of antibiotic (gentamycin: 86.67% and neomycin: 73.33%) (Table 2). All isolates showed multi-drug resistance pattern (MDR). Furthermore, two isolates (sample no 7 and 10) showed the resistance against the highest (5) number of anti-microbial groups (Table 3). Among all the tested isolates resistance determinants, *tet*(A) was the most prevalent (53.85%) and *tet*(C) was the lowest (0%). Both *tet*(A) and *tet*(B) genes were present in 15.38% isolates (Table 4).

Table 4: Result of PCR for detection of *tet* genes in tetracycline-resistant *E. coli* isolates

Gene	Total isolates	Positive sample	Prevalence (%)
tet(A)	13	7	53.85
tet(B)	13	2	15.38
tet(C)	13	0	0
tet(A) + *tet*(B)	13	2	15.38

DISCUSSION

Hospital effluent provides an important environment for the development of multi-drug resistant bacteria. These multi-drug resistant microbes may be released into the aquatic environment resulting in the spread of antibiotic resistance and resistance determinants. Hospitals having no effluent treatment plant (ETP) are mainly responsible for this emerging public health problem. The study revealed that the percentage of *E. coli* in untreated hospital effluent was 100%. Many of the previous studies indicated that the prevalence of *E. coli* varies widely based on type of environmental samples and it was ranging from 44% to 100% in different environmental samples such as sewage, sludge, recreational water, dairy surface water and ground water (Anastasi et al., 2010; Li et al., 2014; Blaak et al., 2014).

The findings of present study is supported by Li et al. (2014) and Blaak et al. (2014) who stated almost similar prevalence of *E. coli* in dairy waste water and recreational water. *E. coli*, isolated from hospital effluent showed highest resistance (ranging from 66% to 100%) against amoxicillin, cephalothin, tetracycline, sulphamethoxazole-trimethoprim, ceftriaxone, nalidixic acid and enrofloxacin.

Highest sensitivity (ranging from 66% to 87%) was found against gentamycin, neomycin and chloramphenicol. These findings showed partial similarity with Katouli et al. (2012) and Shrestha (2012). The isolates showed highest resistant (100%) against β-lactam antibiotics (higher penicillin: amoxicillin and 1st generation cephalosporin) which is agreed by the findings of Katouli et al. (2012) and Shrestha (2012). It may be described due to heavy use of β-lactam antibiotics in last few decades throughout the world. Interestingly, aminoglycosides (gentamycin and neomycin) were highest sensitive in present study which was contradictory to the findings of Katouli et al. (2012). But it showed close agreement with the findings of Ahaduzzaman et al. (2014) and Alam et al. (2006).

Tetracycline resistance was one of the most prevalent types of anti-microbial resistances found in present study and 86.67% isolates were resistant to tetracycline. This finding showed close agreement with Ahaduzzaman et al. (2014) and Barua et al. (2012). Anti-microbial resistance findings of this study were higher than the previous reported results. In the present study 100% isolates showed multi-drug resistance (MDR) pattern. It indicated an increase in upward trend of anti-microbial resistance in pathogens which was agreed with the prediction by Tadesse et al. (2012) who conducted a retrospective study to assess this trend and found statistically significant results. In this study 53.85% isolates were positive for one or more of the tested genes; *tet*(A), *tet*(B) and *tet*(C) whereas 46.15% were negative for all. Findings of Tuckman et al. (2007) showed that 93% of tetracycline-resistant *E. coli* was positive for one or more of the six tested determinant. This variation may be due to variation in number of gene tested and sample size. In present study 53.85%, 15.38% and 0% isolates were positive for *tet*(A) , *tet*(B) and *tet*(C) genes. Again 15.38% isolates possessed both *tet*(A) and *tet*(B) genes which was lower than previous findings; 58% (Tuckman et al., 2007). These studies showed that tetracycline resistance determinant of *E. coli* vary considerably.

The major causes of identified variation may be due to differences in geographical location, strategies of drug administration controlling agencies, anti-microbials used in hospitals etc. In this study a significant proportion of the isolates were negative for all three genes tested, suggesting that these isolates may carry one or more of the other known genes not identified in this study or not previously identified in *E. coli* at all. However, hospital effluent treatment reduces both the total and anti-microbial resistant population of *E. coli* but it doesn't ensure complete elimination of anti-microbial resistant bacterial population in effluent water (Galvin et al., 2010). If the hospitals use effluent treatment plant (ETP), it will reduce the load of anti-microbial resistant *E. coli* in environment.

CONCLUSION

Hospital effluent is one of the major contaminating sources of aquatic environment. Presence of anti-microbial drug residue in the hospital effluent leads to the development of drug resistance in the *E. coli*. The findings of the current study are alarming as infection by anti-microbial resistant *E. coli* will not be recovered following anti-microbial therapy.

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CONFLICT OF INTEREST

Author(s) has no conflict of interest.

AUTHORS' CONTRIBUTION

Dutta A carried out study designing, most of the lab work and manuscript writing. Jalal MS did the PCR and reviewed the manuscript. Nath SK,Dhar PK and Das A collected related data, samples and helped in lab work. Uddin MM supervised all the activities related to this study.

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