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RESEARCH ARTICLE

Presence of *bla*$_{CTXM-1}$, *bla*$_{CTXM-9}$, and *bla*$_{TEM-1}$ Genes in Extended-spectrum β-lactamase-producing *Escherichia coli* Isolates from Hospital Wastewater

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Abstract

BACKGROUND: Extended-spectrum β-lactamase-producing *Escherichia coli* (ESBL-EC) are selectively proliferated in the human gut, excreted through feces, and deposited through wastewater lines, with hospital wastewater acting as a major reservoir of antibiotic resistance genes and resistant bacteria, thus pose adverse effects to human health. This study aimed to determine the presence of *bla*$_{CTXM-1}$, *bla*$_{CTXM-9}$, *bla*$_{TEM-1}$, and *bla*$_{SHV-1}$ genes in ESBL-EC in wastewater from selected hospitals in Manila and Quezon City, the Philippines.

METHODS: Influent and effluent in twelve hospital wastewater treatment plants were collected, screened for cefotaxime-resistant *E. coli*, and examined for the ESBL production through phenotypic characterization using conventional bacterial identification, disk diffusion method, and VITEK® 2 Compact system and genotypic identification of ESBL-EC *bla*$_{CTXM-1}$, *bla*$_{CTXM-9}$, *bla*$_{TEM-1}$, *bla*$_{SHV-1}$ genes using multiplex polymerase chain reaction (PCR).

RESULTS: Conventional bacterial identification methods and the VITEK® 2 Compact system results showed that both influent and effluent samples were positive for ESBL-EC at 33.3% and 16.7%, respectively. Multiplex PCR results revealed that various *E. coli* isolates were of ESBL-EC *bla*$_{CTXM-1}$, *bla*$_{CTXM-9}$, and *bla*$_{TEM-1}$ genes. Multi-drug resistance was observed among all ESBL-EC isolates with resistance being highest against ampicillin, cefuroxime, ceftazidime, ceftriaxone, cefepime, piperacillin, and aztreonam.

CONCLUSION: As the study revealed the presence of ESBL-producing bacteria, efforts must be made to ensure the prudent antimicrobial use with possible emphasis on antibiotic rotation accompanied by intensified infection prevention and control in hospital settings.

KEYWORDS: antimicrobial resistance, beta-lactams, *bla*$_{CTXM}$, *bla*$_{TEM}$, extended-spectrum beta-lactamase, *E. coli*, hospital wastewater

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Introduction

Antimicrobial resistance (AMR) is a major concern to human health contributing to approximately 700,000 deaths annually worldwide from infections due to resistant bacteria. This tally is expected to increase to over 10 million by 2050.(1) The extensive and indiscriminate use of antibiotics in recent years has substantially increased the number of bacterial pathogens resistant to drugs. With fewer antibiotic options available, the emerging difficulty in treating infections is a cause for concern.(2)

Beta (β)-lactams are among the most relevant classes of antibiotics to treat a number of clinically important infections.(3) Pathogenic microorganisms carrying extended-spectrum β-lactamase (ESBL), which have rising prevalence globally, threaten antibiotic therapy.(4) ESBL enzymes cause resistance by deactivating most β-lactams,

particularly penicillin, the first to third generation cephalosporins, and aztreonam through their production of cefotaxime-Munich (CTXM), temoniera (TEM), and sulfhydryl variable (SHV) β-lactamases which are encoded by bla_{CTXM}, bla_{TEM} and bla_{SHV} genes, respectively.(5,6)

Among ESBL-producers are ESBL-producing *Escherichia coli* (ESBL-EC) which are selectively proliferated in the human gut, excreted through feces, and deposited through wastewater lines. The discharge of human waste from sites such as health facilities may thus facilitate the emergence of AMR.(7) bla_{CTXM}, bla_{TEM} and bla_{SHV} genes are the most common isolated gene among the ESBL-EC isolates in Asia. The leading cause of rapid spread is the latent ability of each gene for transmission of resistance genotype that results in a widespread range of contamination.(8) Therefore, wastewater must undergo a treatment process, in which liquid and solid wastes are reduced into stable, non-polluting, and non-infectious matter.(9) When community sanitation and sewage disposal and treatment are not present, efficient, and effective, the chances of community outbreaks due to hospital organisms are extremely high.(10) Thus, this study aimed to determine the antimicrobial susceptibility profile and the presence of bla_{CTXM-1}, bla_{CTXM-9}, bla_{TEM-1}, and bla_{SHV-1} genes in ESBL-EC from wastewater of selected hospitals in Manila and Quezon City, the Philippines.

Methods

Sample Collection and Preparation

This study employed a cross-sectional study design and was conducted in 6 months. Study sites included tertiary hospitals in Manila and Quezon City with a wastewater treatment facility. Upon the approval from the hospital administrations, water samples (50 mL) were collected 5 mm below the surface using "grab sampling" technique in sterile amber glass containers from two sampling points: influent and effluent tanks. Triplicate samples (n=150 mL) from each tank were pooled aseptically, properly labeled, and transported to the laboratory at approximately 5±2°C. Samples were processed within 2 h after collection, maintaining standard procedure.(10) The study protocol has been exempted for review by the Trinity University of Asia-Institutional Ethics Review Committee (No. TUA-IERC-015-R01).

Screening for Lactose-fermenting Colonies

Wastewater samples were processed using serial dilutions and cultured on MacConkey agar supplemented with 2 µg/mL of cefotaxime (CTX) to capture the presumptive ESBL producing bacteria.(11) After overnight incubation at 35°C, lactose-fermenting colonies were selected from each plate sample and subcultured thrice in trypticase soy agar (TSA) for purification. Purified colonies were inoculated in MacConkey and eosin-methylene blue agars.(12,13)

Identification of *E. coli* and ESBL Production With Conventional Method

Colonies of presumptive *E. coli* were Gram stained, inoculated aerobically into triple sugar iron (TSI) agar, sulfide indole motility (SIM) medium, Simmons citrate agar (SCA), lysine iron agar (LIA) slant, and methyl red Voges Proskauer (MRVP) media at 35°C for 24 h.(14,15)

Disk diffusion method on Mueller-Hinton agar (Difco, Franklin Lakes, NJ, USA) was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines.(16) Following an overnight subculture on stock TSA plates, six *E. coli* colonies were inoculated in 5 mL of Mueller-Hinton broth (Oxoid, Hampshire, UK) and incubated for 6 h at 37°C. Each colony was compared to 1.5×10^8 CFU/mL, or the 0.5 McFarland standard. The inoculum was spread on a 25 mL Mueller-Hinton agar plate (Oxoid) using an L-shaped spreader. The plates were incubated at room temperature for approximately 15 min and then antibiotic discs were placed onto the plate surface (≤5 antibiotic discs per plate). The plates were then further incubated for 18 to 24 h at 37°C .(17) ESBL phenotype was determined using antibiotic susceptibility discs (Oxoid) wherein CTX (30 µg) ≤27 mm, ceftazidime (CAZ) (30 µg) ≤22 mm.(18) Results were recorded through measurement of the inhibition zone diameter and interpreted according to standard measurement tables.(16)

Identification of *E. coli* and ESBL Production With VITEK® 2 Rapid Method

Each colony was selected and confirmed up to species-level identification using ID-GNB Card of automated VITEK® 2 Compact system (bioMérieux, Durham, NC, USA).(19,20) The cards were manually put inside the VITEK® 2 reader-inoculator module after being vacuum-inoculated with a 0.5 McFarland suspension of the organism from a sheep blood agar plate that was incubated for 18 to 20 h. Fluorescence was assessed every 15 min for 3 h.(21)

Susceptibility tests were performed using VITEK® 2 system with the 64-well AST-N261 cards in accordance with the manufacturer's instructions and CLSI guidelines.(16) To determine the minimum inhibitory concentration (MIC) of each antibiotic, growth curves were generated using the signal produced every 15 min for 18 h and then

compared to the controls. An algorithm created specifically for each antibiotic was used to perform the calculation. Specifically, CTX and CAZ were used in the ESBL test, either individually (at 0.5 g/mL) and in combination with clavulanic acid (4 g/mL). Once the growth control well has achieved a predetermined threshold (4–18 h of incubation), analysis of all wells was carried out automatically through VITEK® 2 system. The presence of ESBL was shown by a specified decrease in the growth of the CTX or CAZ wells containing clavulanic acid as compared to the level of growth in the well with the cephalosporin alone.(5)

Listed in Table 1 are the different antibiotics used in this study with their Access, Watch, Reserve (AWaRe) classification. This classification is a tool which emphasizes the importance of the appropriate use of various antibiotics.(22)

Six antibiotics were used in disk diffusion while 20 antibiotics were used performing the VITEK® 2 Compact system in accordance with the criteria of the Clinical Laboratory Standards Institute (CLSI) with the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (Table 1).(16,23) For quality assurance of the test, *E. coli* ATCC 25922 and *E. coli* NCTC 13353 were used as negative and positive controls, respectively.(17,24)

DNA Extraction, Concentration, and Purification

Genomic DNA extraction using Presto™ Mini gDNA Bacteria Kit (Macherey-Nagel, Düren, Germany) was performed on all *E. coli* isolates after inoculation in tryptic soy broth (TSB) media and incubation at 37°C for 18 h.(25) NanoDrop spectrophotometer 2000 (Thermo Scientific, Waltham, MA, USA) was used to measure the yield, absorbance and calculate the concentration of nucleic acids (260 nm) and purified proteins (280 nm).(26) The concentration of each selected ESBL-EC isolates were standardized and recorded.

Detection of bla_{CTXM}, bla_{TEM}, and bla_{SHV} Genes

The phenotypically confirmed ESBL-EC were subjected to multiplex polymerase chain reaction (PCR) in order to detect for the presence of bla_{TEM}, bla_{SHV}, and bla_{CTXM} genes.(25) Primer sequences were used for the detection of the ESBL genes (Table 2).(27)

The reaction mixture included a reverse and forward primer (1 µM each), 5 µM Firepol Master Mix (Solis Biodyne, Tartu, Estonia) reaction mixture, DNAse free water, and the DNA template. Amplification was carried out as follows: initial denaturation at 94°C for 10 min; 30 cycles of 94°C for 40 s, 60°C for 40 s and 72°C for 1 min; and a final elongation step at 72°C for 7 min.(27) A 100 bp DNA ladder was used for DNA fragment sizing in agarose gel electrophoresis. No template control was utilized as a negative control, while *E. coli* NCTC 13353 was used as a positive control.(28) The DNA amplicons were observed after running at 100V for 1 h on a 2% agarose gel containing ethidium bromide.(27)

Results

Phenotypic Detection of ESBL-EC

Out of the 12 hospitals, six isolates were identified as *E. coli* by conventional methods (Table 3) and VITEK® 2 Compact system. Four of which were from influent water, while the remaining two were from effluent water. Conventional bacterial identification methods and the VITEK® 2 Compact system results showed that both influent and effluent samples were positive for ESBL-EC at 33.3% and 16.7%, respectively.

All *E. coli* isolates were determined to be ESBL-producing by disk diffusion method and VITEK® 2 Compact system. By disk diffusion method, all isolates were resistant to more than half of the six antibiotics tested with all being resistant against CTX and CAZ (Table 4). By VITEK® 2 Compact system, most isolates presented resistance to more than half of the twenty antibiotics. Resistance was most common to AMP, PIP, CXM, CAZ, CRO, FEP, and ATM, while no resistance to AMK and CST was observed (Table 5). Note that VITEK ® 2 only analyzed specific antibiotic on a certain colony. One major limitation of the VITEK® 2 system in evaluating the susceptibilities of Gram-negative bacteria is its inability to provide the MICs of some agents.

ESBL-encoding Genes (bla_{CTXM}, bla_{TEM} and bla_{SHV}) in *E. coli* from Hospital Wastewater

Out of four *E. coli* isolates from the influent tank which showed phenotypic resistance, two isolates carried the bla_{CTXM} gene (Figure 1A) while three isolates carried the bla_{TEM} gene (Figure 1B). Particularly, there were two isolates (H05 In & H07 In) that carried the bla_{TEM} gene only, while one isolate (H06 In) carried the bla_{CTXM-1} gene only. There was one isolate (H04 In) that carried both bla_{TEM} and bla_{CTXM-1} genes. The bla_{SHV}-type gene was not detected (Figure 1B). One isolate was negative for the three β-lactamase gene primers.

On the other hand, both ESBL-EC isolates from the effluent tank carried the bla_{CTXM} gene, with one isolate (H06 eff) carrying the bla_{CTXM-1} gene and the other (H01 eff) bla_{CTXM-9} gene (Figure 1C).

Table 1. Antibiotic assay discs abbreviations and amount of antibiotic contained in each disc.

Antibiotic Discs	Abbreviations	Concentration	AWaRe Classification (WHO 2021)
For disc diffusion assay			
Cefoxitin (2nd generation Cephalosporin)	FOX	30 µg	Watch
Cefotaxime (3rd generation Cephalosporin)	CTX	30 µg	Watch
Ceftazidime (3rd generation Cephalosporin)	CAZ	30 µg	Watch
Cefepime (4th generation Cephalosporin)	FEP	30 µg	Watch
Gentamicin (Aminoglycoside)	GEN	30 µg	Access
Ciprofloxacin (Fluoroquinolone)	CIP	5 µg	Watch
For VITEK® 2 compact system			
Ampicillin (Penicillin)	AMP	10 µg	Access
Amoxicillin (Penicillin)	AMX	30 µg	Access
Piperacillin (Penicillin)	PIP	30 µg	Watch
Piperacillin/Tazobactam (Penicillin)	TZP	30 µg	Watch
Cefuroxime (2nd generation Cephalosporin)	CXM	30 µg	Watch
Cefoxitin (2nd generation Cephalosporin)	FOX	30 µg	Watch
Ceftazidime (3rd generation Cephalosporin)	CAZ	30 µg	Watch
Ceftriaxone (3rd generation Cephalosporin)	CRO	30 µg	Watch
Cefepime (4th generation Cephalosporin)	FEP	30 µg	Watch
Aztreonam (Monobactam)	ATM	30 µg	Reserve
Ertapenem (Carbapenem)	ETP	30 µg	Watch
Imipenem (Carbapenem)	IPM	30 µg	Watch
Meropenem (Carbapenem)	MEM	30 µg	Watch
Amikacin (Aminoglycoside)	AMK	30 µg	Access
Gentamicin (Aminoglycoside)	GEN	10 µg	Access
Ciprofloxacin (Fluoroquinolone)	CIP	30 µg	Watch
Levofloxacin (Fluoroquinolone)	LVX	30 µg	Watch
Ofloxacin (Fluoroquinolone)	OFX	30 µg	Watch
Colistin (Polymyxin)	CST	30 µg	Reserve
Trimethoprim/Sulfamethoxazole (Folate Synthesis Inhibitor)	SXT	30 µg	Access

Discussion

ESBL-EC isolates were identified in six sewage sites of 12 hospitals. In comparison to 2021 national data, the positivity rate of *E. coli* isolates (n=7,404) for ESBL production was 24.51%. A retrospective study revealed an increasing trend of ESBL from 1999-2013.(29) In terms of resistance, all ESBL-EC isolates were resistant to AMP, CXM, CAZ, CRO, and ATM which was similar to the results of a local

Table 2. Summary of ESBL-producing *E. coli* primer sequences for multiplex PCR.

PCR Target	Primer Name	Primer Sequence (5'- 3')	Amplicon Size (bp)
bla $_{TEM-1}$	MultiTSO-T_for	CATTTCCGTGTCGCCCTTATTC	800
	MultiTSO-T_rev	CGTTCATCCATAGTTGCCTGAC	
bla $_{CTXM-1}$	MultiCTXMGp1_for	TTAGGAARTGTGCCGCTGYA	688
	MultiCTXMGp1_rev	CGATATCGTTGGTGGTRCCAT	
bla $_{CTXM-9}$	MultiCTXMGp9_for	TCAAGCCTGCCGATCTGGT	561
	MultiCTXMGp9_rev	TGATTCTCGCCGCTGAAG	
bla $_{SHV-1}$	MultiTSO-S_for	AGCCGCTTGAGCAAATTAAAC	713
	MultiTSO-S_rev	ATCCCGCAGATAAATCACCAC	

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Table 3. Results of isolates by manual Gram reaction, colony morphology, and biochemical reaction.

Hospital	Influent/ Effluent	Gram Reaction	Mac	EMB	Indole	MR	VP	SCA	Motility	TSI	LIA (Interpretation)	LIA (Result)	Possible Organism
H01	Effluent	Negative	Pink, creamy colonies	Green metallic sheen colonies	(+)	(+)	(-)	(-)	Motile	A/A w/ Gas	LDC (-) / LDA (-)	K/A	*E. coli*
H04	Influent	Negative	Pink, creamy colonies	Green metallic sheen colonies	(+)	(+)	(-)	(-)	Motile	A/A w/ Gas	LDC (-) / LDA (-)	K/A	*E. coli*
H05	Influent	Negative	Pink, creamy colonies	Green metallic sheen colonies	(+)	(+)	(-)	(-)	Motile	A/A w/ Gas	LDC (-) / LDA (-)	K/A	*E. coli*
H06	Influent	Negative	Pink, creamy colonies	Green metallic sheen colonies	(+)	(+)	(-)	(-)	Motile	A/A w/ Gas	LDC (-) / LDA (-)	K/A	*E. coli*
	Effluent	Negative	Pink, creamy colonies	Green metallic sheen colonies	(+)	(+)	(-)	(-)	Motile	A/A w/ Gas	LDC (-) / LDA (-)	K/A	*E. coli*
H07	Influent	Negative	Pink, creamy colonies	Green metallic sheen colonies	(+)	(+)	(-)	(-)	Motile	A/A w/ Gas	LDC (-) / LDA (-)	K/A	*E. coli*

Mac: MacConkey agar; EMB: Eosin-methylene blue; MR: Methyl red; VP: Voges Proskauer; SCA: Simmons citrate agar; TSI: Triple sugar iron agar; LIA: Lysine iron agar; A/A: Acid slant/acid butt; LDC: Lysine decarboxylase; LDA: Lysine deaminase; K/A: Alkaline slant/alkaline butt.

Table 4. Resistance profiles of *E. coli* identified by disk diffusion of influent and effluent water, December 2019.

Hospital	Influent/ Effluent	Bacteria	Antibiotic					
			FOX	CTX	CAZ	FEP	GEN	CIP
H01	In	No *E. coli* detected						
	Eff	*E. coli*	R	R	R	R	S	S
H04	In	*E. coli*	R	R	R	R	R	R
	Eff	No *E. coli* detected						
H05	In	*E. coli*	R	R	R	R	I	R
	Eff	No *E. coli* detected						
H06	In	*E. coli*	S	R	R	R	S	R
	Eff	*E. coli*	R	R	R	S	R	S
H07	In	*E. coli*	R	R	R	R	R	S
	Eff	No. *E. coli* detected						

In: Influent; Eff: Effluent; S: Susceptible; I: Intermediate; R: Resistant.

study where ESBL-producing bacteria showed the highest resistance to AMP (100%), CTX (79.3–100%), CAZ (35–100%), and ATM (87–94%).(29)

The findings of the study revealed that all ESBL-EC presented multidrug resistance which is common for these organisms as plasmids that carry genes that code for ESBLs also commonly contain other genes that encode for mechanisms of resistance to other antimicrobial drugs such as quinolones, aminoglycosides, chloramphenicol. (30,31) Therefore, infections caused by ESBL-producing Enterobacteriaceae are difficult to treat which may entail a significant increase in the burden of nosocomial infections.(32)

Moreover, resistance was observed against each "Access" group antibiotic with the exception of AMK. The Access group includes essential first or second choice antibiotics that have activity against a wide range of pathogens while presenting the lowest potential for resistance than other antibiotics.(22) On the other hand, at least one isolate conferred resistance against each of the included "Watch" group antibiotic.(22) The Watch group includes antibiotics with a higher potential for resistance and contains most of the highest-priority agents among Critically Important Antimicrobials for Human Medicine.(22) For "Reserve" group antibiotics, high resistance was observed against ATM while all isolates were susceptible to CST. When all other treatments have failed or are inappropriate, the Reserve group of antibiotics should be used as a "last resort" option for very specific people and situations.(22) Drug resistance usually entails prolonged hospital stays and increased treatment costs, with the increased risk of treatment failure from inappropriate antibiotic therapy. (31) It is also concerning that the detected organisms were resistant to CIP and GEN which are considered alternative treatment regimens to carbapenems.(31)

The production of β-lactamase enzymes among Enterobacteriaceae is driven by selective pressure due to many interacting factors including clinical and environmental factors, human activities, and indiscriminate use of antimicrobials. This highlights the requirement for more stringent hospital policies to maximize the proper use of antibiotics and reduce antibiotic resistance (33,34) with emphasis on the appropriate use of third to fourth generation cephalosporins and possibly antibiotic cycling. Its components must include: 1) multidisciplinary coordination between hospital administrators, clinicians, infectious disease specialists, infection control teams, microbiologist, hospital pharmacist; 2) regulation of prescription by consultant specialist; 3) monitoring and auditing of drug use; 4) surveillance and reporting of resistance patterns of the hospital flora; and 5) proper management of hospital specially during transit of patients.(8,34,35) Surveillance for nosocomial infections by ESBL-producing organisms may be focused on oncology, burns, intensive care units and neonatal wards where these are commonly associated.(31)

Many studies in clinical and/or environmental settings showed that among the three ESBL types, the dominating type is the bla_{CTXM} gene while others found bla_{SHV} as the most prevalent.(28,32,36,37) Still, other studies have revealed that bla_{SHV} was the least common among Enterobacteriaceae.(38) In this study, the environmental wastewater samples did not reveal the bla_{SHV} gene. The differences in the predominant type of ESBLs are understandable as they may vary per

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Table 5. Resistance profiles of *E. coli* identified by VITEK® 2 Compact system of influent and effluent water, December 2019.

Hospital	Influent/ Effluent	Bacteria	Antibiotic																			
			AMP	AMX	PIP	TZP	CXM	FOX	CAZ	CRO	FEP	ATM	ETP	IPM	MEM	AMK	GEN	CIP	LVX	OFX	CST	SXT
H01	In	No *E. coli* detected																				
	Eff	*E. coli*	R	R	R	R	R	R	R	R	R	R	R	S	R	S	S	S	S	S	S	S
H04	In	*E. coli*	R	R	R		R	R	R	R	R	R	S	S	S	S	R	R	R	R	S	R
	Eff	No *E. coli* detected																				
H05	In	*E. coli*	R	R		R	R	R	R	R	R		R	R	R	S	I	R	R	R	S	R
	Eff	No *E. coli* detected																				
H06	In	*E. coli*	R	S	R	S	R	S	R	R	R	R	S	S	S	S	R	R	R	R	S	R
	Eff	*E. coli*	R	R		S	R	S	R	R	R	R	S	S	S	S	R	S	S	S	S	S
H07	In	*E. coli*	R	R	R	S	R	R	R	R	R	R	S	S	S	S	R	S	S	S	S	S
	Eff	No. *E. coli* detected																				

In: Influent; Eff: Effluent; S: Susceptible; I: Intermediate; R: Resistant.

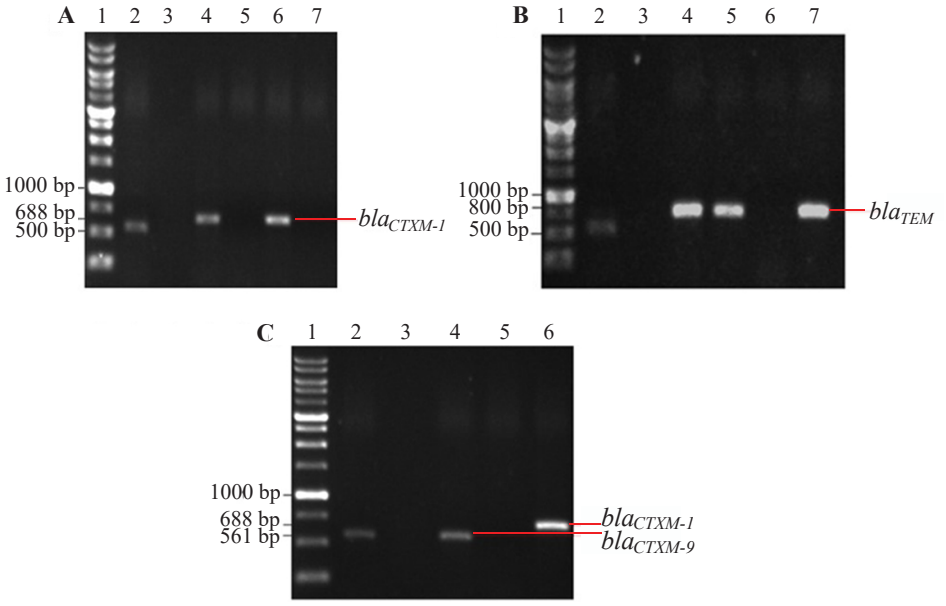

Figure 1. Multiplex PCR assay for the detection of *bla*$_{CTXM}$, *bla*$_{TEM}$, and *bla*$_{SHV}$ genes. A: Multiplex PCR assay for the detection of *bla*$_{CTXM}$ gene in influent wastewater tanks. Lanes: 1, Ladder; 2, *E. coli* NCTC 13353; 3, negative control; 4, *E. coli* isolate in Hospital 4; 5, *E. coli* isolate in Hospital 5; 6, *E. coli* isolate in Hospital 6; 7, *E. coli* isolate in Hospital 7; B: Multiplex PCR assay for the detection of *bla*$_{TEM}$ and *bla*$_{SHV}$ genes in influent wastewater tanks. Lanes: 1, Ladder; 2, *E. coli* NCTC 13353; 3, negative control; 4, *E. coli* isolate in Hospital 4; 5, *E. coli* isolate in Hospital 5; 6, *E. coli* isolate in Hospital 6; 7, *E. coli* isolate in Hospital 7; C: Multiplex PCR assay for the detection of *bla*$_{CTXM}$ gene in effluent wastewater tanks. Lanes: 1, Ladder; 2, *E. coli* NCTC 13353; 3, negative control; 4, *E. coli* isolate in Hospital 1; 5, excluded isolate; 6, *E. coli* isolate in Hospital 6.

location.(8) Generally social, health, and environmental factors have been identified to be connected to variations in the abundance of AMR genes.(39)

In contrast with the results in the untreated wastewater, findings in the treated wastewater agree with more studies, in which the *bla*$_{CTXM}$ gene was the predominant type.(28,32,36) Considering its widespread presence in China and India, *bla*$_{CTXM}$ genes have been speculated to be the most frequent type worldwide.(40)

Wastewater is treated to make it suitable for reuse and release into the environment without harming the ecosystem. In terms of water treatment, all hospitals in the study employed only chlorination with most disposing their effluent to drainage and one disposing to a river. Only two hospitals recycled their effluents. Although chlorination can kill bacteria, the antimicrobial resistance gene may survive and spread to different bacteria via horizontal gene transfer from a biofilm in the effluent tank. The ESBL resistance gene of *E. coli* has been considered genetically diverse and highly mobile. This may explain why ESBL-EC was detected in effluent despite absence in the influent. As ESBL-EC organisms were still observed in the effluents of two hospitals, there is a need to strengthen the implementation of efficient water treatment facilities across all hospitals. (40) Further, there is the need to study the content of ESBL-EC in wastewater recycling to determine safety before disposing into sewers or into rivers.

Additionally, the findings of the study also highlight the need for environmental surveillance.(34) Several studies have documented the widespread occurrence of AMR genes in hospital wastewater in spite of treatment which contributes to the spread of these emerging pollutants in the environment. Resistance may further develop in the environment when these organisms are mixed with other waste and chemicals, which may propagate freely to find its way back to humans.(28,32,36)

According to Philippine Clean Water Act of 2005, hospitals should provide water utilities for septage management services. Local Government Units (LGUs) are required to offer such services in the absence of a water utility, either independently or under a service agreement. In some cases, private organizations ought to offer these services in place of or concurrently with LGU or water utility activity. Sewage management is a practical first step for most utilities and LGUs because sewerage systems are scarce and expensive to build and run. The National Building Code of the Philippines (RA 6541) and the Revised National Plumbing Code of the Philippines also have regulations addressing proper septic tank design, operation, and maintenance, in addition to the legislation mentioned.

Septage management includes comprehensive programs for managing septic tank and wastewater treatment. A comprehensive septage management program includes septic tank design and construction; septic tank inspection which include testing of wastewater samples for the presence of pathogenic microorganism such as antibiotic resistant bacteria and other operating conditions (pH, free available chlorine, hydraulic retention time, solid retention time, biomass concentration) every month; procedures for septic tank desludging and septage transportation; record keeping and reporting; and septage disposal. Thus, there is a need for strengthened collaboration with the environmental sector whose role in combating AMR has long been recognized through the One Health approach.(34)

Increasing monitoring and surveillance for these microorganisms complemented by timely reporting and feedback must also be given importance to detect possible outbreaks in clinical settings. Environmental surveillance must also be institutionalized to monitor the emergence of other resistant organisms of public health concern.

Conclusion

The study showed the presence of ESBL-producing bacteria. Both influent and effluent wastewater samples may be a source of ESBLs, antibiotic resistance bla_{CTXM-1}, bla_{CTXM-9}, bla_{TEM-1} and present a potential environmental health risk. As the study revealed a high positivity rate of ESBL-producing *E. coli*, efforts must be made to ensure the prudent use of antimicrobials with possible emphasis on antibiotic rotation accompanied by intensified infection prevention and control in hospital settings.

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Authors Contribution

CPST and GBD equally contributed in conceptualizing the research, performing sample collection and data analysis, preparing the manuscript, designing the figures and tables, and interpreting the results. Both authors took part in giving critical revision of the manuscript.

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