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Persistence of Antibiotic-Resistant *Escherichia coli* Following Chlorine Disinfection of Hospital Wastewater

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ABSTRACT

Background and Aim: This study investigated the prevalence of antibiotic-resistant *Escherichia coli* in untreated and treated wastewater from a hospital wastewater treatment system and assessed their persistence after chlorine disinfection.

Materials and Methods: The study included three main steps. First, *E. coli* was isolated from untreated and treated wastewater samples. Second, antibiotic susceptibility was assessed phenotypically. Ten antibiotics were tested using the Kirby–Bauer disk diffusion method, while colistin susceptibility was evaluated by broth microdilution to determine the minimum inhibitory concentration (MIC). Colistin results were interpreted according to the EUCAST breakpoint for Enterobacterales, with MIC ≤2 mg/L considered susceptible and MIC >2 mg/L considered resistant. Third, selected resistance genes related to ESBL production, colistin resistance, and carbapenem resistance were detected by PCR after thermal DNA extraction.

Results: *Escherichia coli* was detected in 5 of 10 untreated wastewater samples (50.0%) and 6 of 10 treated wastewater samples (60.0%), with no statistically significant difference ($p > 0.05$). Because the sample size was small and more than one isolate was obtained from some samples, the results should be interpreted as detection-frequency data rather than evidence of bacterial-load reduction. Ampicillin resistance was the most common phenotype in untreated wastewater isolates (86.7%) and was detected in all treated wastewater isolates (100%). Fosfomycin resistance was the least common phenotype in treated wastewater isolates (5.6%). The genes *bla*TEM, *bla*CTX-M-1 group, *bla*CTX-M-9 group, and *mcr*-1 were detected in isolates from both wastewater types, while selected carbapenemase genes were detected in treated-wastewater isolates.

Conclusion: The treatment system reduced conventional physicochemical pollutants but did not fully remove detectable antibiotic-resistant *E. coli* or selected resistance genes.

Keywords: *Escherichia coli*, Antibiotic Resistance Genes, Hospital Wastewater, Chlorination, Antimicrobial Resistance

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1. Introduction

Antimicrobial resistance is a major global public health concern. Recent reports estimate that multidrug-resistant bacterial infections cause a substantial number of deaths each year, and this burden is expected to increase markedly in the coming decades if effective control measures are not implemented **(1–3)**. Antibiotic-resistant bacteria (ARBs) and antibiotic resistance genes (ARGs) can spread through different routes, including mobile genetic elements such as plasmids, integrons, and transposons **(4–8)**. This is particularly important for Gram-negative bacteria, including *Escherichia coli*, because many resistance determinants can be transferred horizontally between bacterial populations.

Carbapenem-resistant Enterobacteriaceae have been recognized by the World Health Organization (WHO) as a serious threat because of their association with limited treatment options, high mortality, and potential for rapid dissemination **(9)**. Carbapenem resistance is commonly mediated by carbapenemase enzymes encoded by different *bla* genes, including enzymes belonging mainly to Ambler classes A, B, and D **(10–13)**. In addition, extended-spectrum β-lactamase (ESBL)-producing *E. coli* has become an important target for surveillance within a One Health framework. In 2021, WHO proposed integrated monitoring of ESBL-producing *E. coli* across humans, animals, food, and the environment to better understand the spread of antimicrobial resistance and support national surveillance strategies **(14, 15)**.

In Viet Nam, antibiotic-resistant bacteria have been reported in food, environmental sources, and clinical samples **(16–19)**. ESBL-producing Enterobacterales are common, with TEM, SHV, and CTX-M enzymes among the main β-lactamase types detected in Gram-negative bacteria **(17, 20, 21)**. Colistin-resistant and *mcr*-positive *E. coli* have also become a concern, with reports from animals, food, clinical specimens, and healthy carriers **(22–25)**. Because *mcr* genes are often located on mobile genetic elements, they may contribute to the spread of colistin resistance between bacterial populations.

Hospital wastewater is an important environmental reservoir for antimicrobial resistance. Hospitals use large amounts of antibiotics, and their wastewater may contain antibiotic residues, resistant bacteria, and resistance genes. If wastewater treatment is insufficient, these contaminants may enter surrounding aquatic environments and contribute to the wider dissemination of antimicrobial resistance **(26–30)**. This concern is particularly relevant in developing countries, where hospital wastewater treatment systems may vary in performance and routine monitoring of ARBs and ARGs is often limited.

Sa Dec General Hospital in Dong Thap Province, Viet Nam, uses chlorine disinfection before wastewater discharge. However, the ability of chlorination to eliminate antibiotic-resistant *E. coli* and selected resistance genes under routine operating conditions remains unclear. Therefore, this study aimed to evaluate the occurrence of antibiotic-resistant *E. coli* and selected resistance genes in untreated and chlorine-treated wastewater from Sa Dec General Hospital. The findings may help support routine monitoring and improve control strategies for antimicrobial resistance in hospital wastewater systems.

2. Materials and Methods

2.1 Sampling locations and procedures in the wastewater treatment process

Dong Thap is a province in the Mekong Delta region of southern Viet Nam, with an area of 3,384 km² and a population of approximately 1.7 million in 2025, accounting for 9.19% of the Mekong Delta population **(31)**. The study was conducted at Sa Dec General Hospital, Dong Thap Province, Viet Nam. The hospital has more than 300 beds, with nine departments and 19 clinical and subclinical units.

A total of 20 wastewater samples were collected between January 2023 and October 2023. These included 10 untreated wastewater samples and 10 chlorine-treated wastewater samples. Sampling was performed according to ISO 5667-10:2020 guidelines for wastewater sampling **(32)**. Untreated samples were collected from the receiving tank before treatment, while treated samples were collected from the discharge point after chlorination.

As shown in Figure 1, wastewater first entered the receiving tank, where large solids were removed. It then passed into the equalization tank, which stabilized flow and pollutant levels before biological treatment. In the aerotank, aerobic microorganisms degraded organic matter. The wastewater then moved to the settling tank, where biomass was separated from the clarified water. The clarified effluent was disinfected with chlorine at approximately 25 mg/L for 30 min before discharge. Residual chlorine in the treated effluent ranged from 0.85 to 0.95 mg/L.

Treated wastewater samples were collected in sterile containers containing sodium thiosulfate to neutralize residual chlorine and prevent continued

disinfectant activity after sampling. Samples were transported to the laboratory under cold conditions and processed as soon as possible after collection.

During evaluation of the chlorination process, pH, total suspended solids (TSS), chemical oxygen demand (COD), biochemical oxygen demand (BOD_5, measured after 5 days at 20 °C), and residual chlorine in the treated effluent were monitored before and after treatment. These parameters were used to assess the physicochemical performance of the wastewater treatment system before environmental discharge.

2.2 Phenotypic and genotypic analysis of antibiotic-resistant *E. coli*

Hospital wastewater samples were analyzed in three main steps: isolation of *E. coli*, phenotypic antimicrobial susceptibility testing, and PCR-based detection of selected antibiotic resistance genes (Figure 2).

2.3 Isolation of *E. coli*

For the presumptive isolation of *E. coli*, 100 mL of each wastewater sample was filtered through a 0.45 µm membrane filter. The membrane was then placed on chromogenic coliform agar (CCA; Merck, Germany), with care taken to avoid air bubbles beneath the membrane, and incubated at 44 °C for 24 h. Colonies showing dark green to purple coloration were recorded as presumptive *E. coli* according to the applied culture method **(33-35)**.

2.4 Phenotypic antibiotic resistance

Antimicrobial susceptibility testing was performed for ampicillin, amoxicillin/clavulanic acid, cefazolin, imipenem, gentamicin, tetracycline, ciprofloxacin, sulfamethoxazole-trimethoprim, chloramphenicol, and fosfomycin using the Kirby–Bauer disk diffusion method in accordance with CLSI (2023) guidance. Colistin susceptibility was tested separately by broth microdilution in cation-adjusted Mueller–Hinton broth to determine the minimum inhibitory concentration (MIC), because disk diffusion is not reliable for colistin testing. Colistin MIC results were interpreted using the EUCAST breakpoint for Enterobacterales, with MIC ≤2 mg/L considered susceptible and MIC >2 mg/L considered resistant **(36)**.

2.5 PCR-based detection of resistance genes

Presumptive *E. coli* isolates were cultured at 35 °C on Tryptic Soy Agar (TSA; Merck, Germany). Colonies were transferred into Eppendorf tubes containing 500 µL of TE buffer (Invitrogen, USA). The tubes were boiled at 95 °C for 10 min, cooled on ice for 5 min, and centrifuged at 10,000 rpm for 10 min. The supernatant was stored at −20 °C until PCR analysis **(37)**. Selected resistance genes associated with β-lactam resistance, colistin resistance, and carbapenem resistance were detected by PCR. The target genes, primer sequences, and PCR conditions are shown in Tables 1 and 2. Because group-specific CTX-M primers were used, CTX-M results were reported as *bla*CTX-M-1 group, *bla*CTX-M-2 group, *bla*CTX-M-9 group, or *bla*CTX-M-8/25 group, rather than as specific CTX-M alleles.

2.6. Statistical analysis

Statistical analyses were performed using IBM SPSS Statistics (IBM Corp., Armonk, NY, USA). Physicochemical parameters, including pH, total suspended solids (TSS), biochemical oxygen demand (BOD_5), and chemical oxygen demand (COD), are presented as mean ± standard deviation (SD). The normality of each variable was assessed using the Shapiro–Wilk test. Untreated and treated wastewater samples were compared using an independent-samples *t*-test when data were normally distributed; otherwise, the Mann–Whitney U test was used.

The detection frequency of presumptive *E. coli* in untreated and treated wastewater samples was compared on a per-sample basis using Fisher's exact test. Antibiotic resistance proportions were also compared between isolates from untreated and treated wastewater using Fisher's exact test for each antibiotic. However, because multiple isolates were recovered from some wastewater samples, isolates from the same sample were not considered fully independent. Therefore, isolate-level resistance comparisons and their associated *p*-values were interpreted as exploratory and not as confirmatory evidence of treatment-associated selection. A two-sided *p* value <0.05 was considered statistically significant.

Table 1. Nucleotide sequences of primers for detecting ARGs.

Class	Target gene	Sequence (5′–3′)	Product size (bp)	Reference
GAPDH	TEM-1-F	GGTCGCCGCATACACTATTCTC	372	Le et al **(16)**
	TEM-1-R	TTTATCCGCCTCCATCCAGTC		
	SHV-F	CCAGCAGGATCTGGTGGACTAC	231	
	SHV-R	CCGGGAAGCGCCTCAT		
	CTX-M-1-F	GAATTAGAGCGGCAGTCGGG	588	
	CTX-M-1-R	CACAACCCAGGAAGCAGGC		
	CTX-M-2-F	GATGGCGACGCTACCCC	107	
	CTX-M-2-R	CAAGCCGACCTCCCGAAC		
	CTX-M-9-F	GTGCAACGGATGATGTTCGC	475	
	CTX-M-9-R	GAAACGTCTCATCGCCGATC		
	CTX-M-8/25-F	GCGACCCGCGCGATAC	186	
	CTX-M-8/25-R	TGCCGGTTTTATCCCCG		
Polymyxin B (colistin)	*mcr-1*	ATGCCAGTTTCTTTCGCGTG	502	Than et al **(25)**
	mcr-1	TCGGCAAATTGCGCTTTTGGC		
	mcr-2	GATGGCGGTCTATCCTGTAT	379	
	mcr-2	AAGGCTGACACCCCATGTCAT		
	mcr-3	ACCAGTAAATCTGGTGGCGT	296	
	mcr-3	AGGACAACCTCGTCATAGCA		
	mcr-4	TTGCAGACGCCCATGGAATA	207	
	mcr-4	GCCGCATGAGCTAGTATCGT		
	mcr-5	GGACGCGACTCCCTAACTTC	608	
	mcr-5	ACAACCAGTACGAGAGCACG		
Carbapenem	bla_{OXA48}	TTGGTGGCATCGATTATCGG	744	Albiger et al **(38)**
	bla_{OXA48}	GAGCACTTCTTTTGTGATGGC		
	bla_{VIM}	AGTGGTGAGTATCCGACAG	212	
	bla_{VIM}	TCAATCTCCGCGAGAAG		
	bla_{NDM}	TGGCAGCACACTTCCTATC	488	
	bla_{NDM}	AGATTGCCGAGCGACTTG		
	bla_{KPC}	CTGTCTTGTCTCTCATGGCC	796	
	bla_{KPC}	CCTCGCTGTRCTTGTCATCC		

Table 2. PCR reaction components and thermal cycling conditions for beta-lactamase, colistin-resistance, and carbapenemase genes.

Class	Quantity	Final concentration	Volume of aspirated (µL)	Thermal cycle
Beta-lactamase	Gotaq Green Master Mix 2X	1X	9	95 °C/5 min; 25 cycles x (95 °C /30 s, 60 °C /90 s, 72 °C / 90 s); 68 °C/10 min
	Forward and reverse primer	10 µM	0.6/ each primer	
	DNA	10-50 nM	1.0	
	Nuclease-free water		2.8	
	Total		20	
Polymyxin B (colistin)	Gotaq Green Master Mix 2X	1X	10	94 ºC/4 min; 30 cycles x (94 ºC/50 s, 59 ºC/20 s); 72 ºC/5 min; 4 ºC
	mcr-1, mcr-2, mcr-3	20 µM	1,0/ each primer	
	mcr-4, mcr -5	20 µM	0.5/ each primer	
	DNA	10-50 nM	1.0	
	Nuclease-free water		1.0	
	Total		20	
Carbapenem	Gotaq Green Master Mix 2X	1X	10	94 ºC/5 min; 30 cycles x (94 ºC/30 s, Tm*/30 s, 72 ºC/1 min); 72 ºC/10 min
	Forward primer	10 µM	0.5	
	Reverse primer	10 µM	0.5	
	DNA	10-50 nM	1.0	
	Nuclease-free water	1X	8.0	
	Total		20	

***Melting temperature (Tm): bla_{VIM}: 52 °C; bla_{OXA48} and bla_{NDM}: 58 °C; bla_{KPC}: 60 °C**

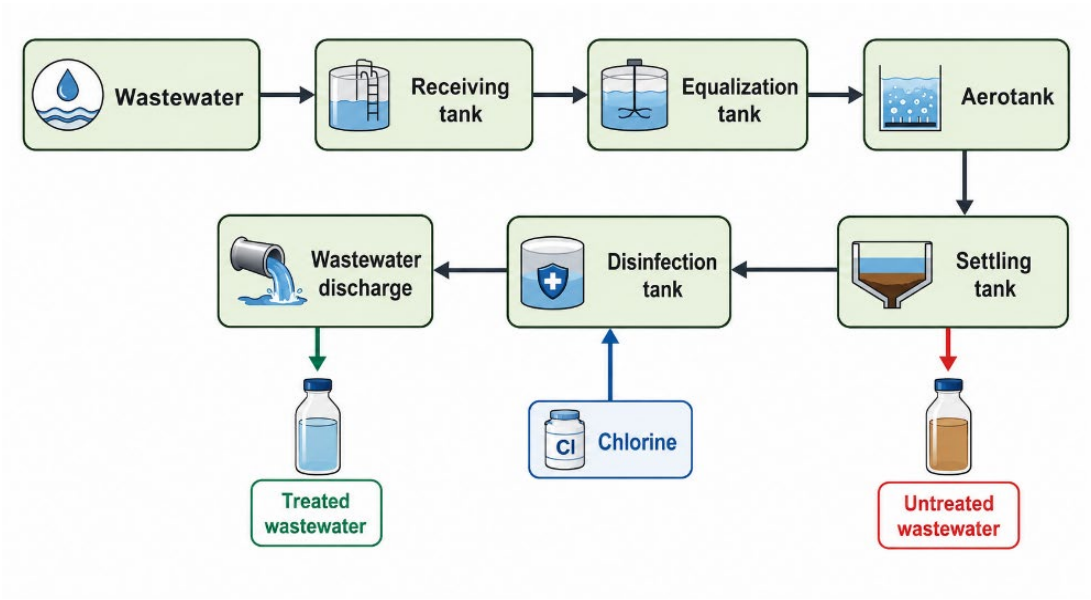

Figure 1. Sampling locations and procedures in the wastewater treatment process **(Prepared by Authors, 2026)**.

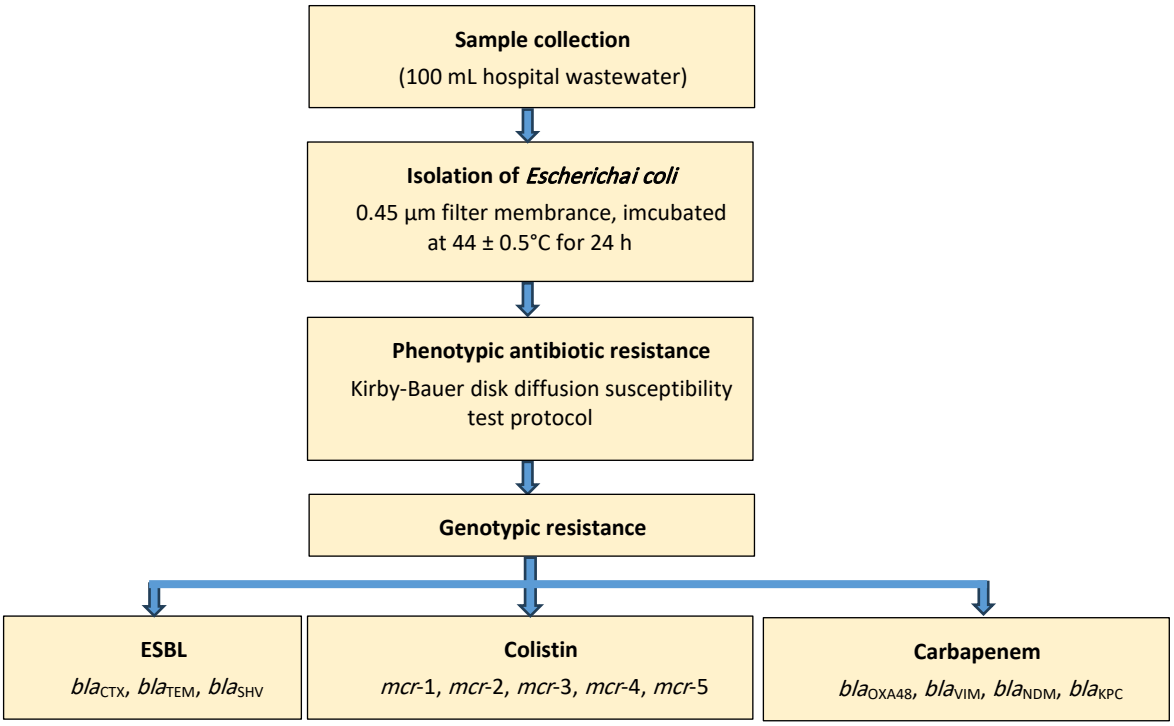

Figure 2. The process of analyzing hospital wastewater samples **(Prepared by Authors, 2026)**.

3. Results

3.1 Isolation of *E. coli* strains

In total, 33 presumptive *Escherichia coli* isolates were recovered from the 20 wastewater samples. Of these, 15 isolates were obtained from untreated wastewater and 18 from treated wastewater. Because more than one isolate was recovered from some samples, the number of isolates was higher than the number of positive samples. On a per-sample basis, presumptive *E. coli* was detected in 5 of 10 untreated wastewater samples (50.0%) and 6 of 10 treated wastewater samples (60.0%). This difference was not statistically significant by Fisher's exact test ($p > 0.05$). Under the conditions of this study, chlorination did not show a measurable reduction in the frequency of culture-positive samples. However, these findings should not be interpreted as evidence of bacterial-load reduction, because colony counts or concentration-based removal efficiencies were not determined.

3.2 Physicochemical characteristics of untreated and treated wastewater

The physicochemical parameters of untreated and treated wastewater were analyzed and reported as mean ± SD. The pH values did not differ significantly between untreated and treated wastewater ($p > 0.05$). In contrast, TSS, BOD_5, and COD were significantly lower in treated wastewater than in untreated wastewater ($p < 0.001$), indicating effective removal of conventional physicochemical pollutants by the treatment system (Table 3).

3.3. Antibiotic resistance phenotype of *E. coli* isolates

Antibiotic resistance phenotypes were determined for all 33 presumptive *E. coli* isolates, and the results are summarized in Table 4. Among isolates from untreated wastewater, resistance was most frequently observed against ampicillin (13/15; 86.7%), followed by tetracycline (12/15; 80.0%) and colistin (10/15; 66.7%). Resistance to cefazolin, sulfamethoxazole-trimethoprim, chloramphenicol, and fosfomycin was detected in 8 of 15 isolates (53.3%). Resistance to ciprofloxacin, gentamicin, and amoxicillin/clavulanic acid was found in 7/15 (46.7%), 5/15 (33.3%), and 4/15 (26.7%) isolates, respectively. No imipenem-resistant isolate was detected in untreated wastewater.

Among isolates from treated wastewater, all 18 isolates were resistant to ampicillin (100%). High resistance rates were also observed for cefazolin (17/18; 94.4%), tetracycline (13/18; 72.2%), ciprofloxacin and chloramphenicol (12/18; 66.7% each), gentamicin (11/18; 61.1%), and sulfamethoxazole-trimethoprim (9/18; 50.0%). Lower resistance rates were observed for amoxicillin/clavulanic acid (7/18; 38.9%), colistin (6/18; 33.3%), imipenem (4/18; 22.2%), and fosfomycin (1/18; 5.6%).

At the isolate level, Fisher's exact test suggested a higher frequency of cefazolin resistance and a lower frequency of fosfomycin resistance among treated-wastewater isolates. However, because several isolates

were recovered from the same wastewater sample, these comparisons were considered exploratory. Therefore, the observed differences should not be interpreted as confirmatory evidence of treatment-associated selection. The recovery of imipenem-resistant isolates only from treated wastewater should also be interpreted cautiously and considered together with carbapenemase-gene detection results.

These findings show that antibiotic-resistant *E. coli* remained detectable in treated hospital wastewater. Although chlorination was applied at an initial dose of 25 mg/L for 30 min and residual chlorine ranged from 0.85 to 0.95 mg/L, resistant isolates were still recovered after treatment. This suggests that chlorination alone may not be sufficient to completely eliminate antimicrobial-resistant *E. coli* under the conditions evaluated. Strengthening antimicrobial stewardship in healthcare settings and improving wastewater treatment barriers may help reduce the environmental release of multidrug-resistant bacteria.

3.4 Resistance genes for β-lactams, carbapenems, and colistin in *E. coli*

Based on phenotypic testing, 6 of 15 isolates from untreated wastewater and 12 of 18 isolates from treated wastewater showed a presumptive ESBL phenotype (Table 5). Among isolates from untreated wastewater, three carried the *bla*CTX-M-1 group, one carried both *bla*TEM and the *bla*CTX-M-9 group, and one carried the *bla*CTX-M-9 group alone. The *bla*SHV, *bla*CTX-M-2 group, and *bla*CTX-M-8/25 group genes were not detected in untreated-wastewater isolates (Figure 3).

Among isolates from treated wastewater with a presumptive ESBL phenotype, five carried both *bla*TEM and the *bla*CTX-M-1 group, two carried *bla*TEM alone, three carried both *bla*TEM and the *bla*CTX-M-9 group, and two carried the *bla*CTX-M-1 group alone. Similar to untreated wastewater, *bla*SHV, *bla*CTX-M-2 group, and *bla*CTX-M-8/25 group genes were not detected in treated-wastewater isolates.

For colistin resistance, *mcr-1* was detected in 10 of 15 untreated-wastewater isolates and 6 of 18 treated-wastewater isolates (Figure 4). Other tested *mcr* genes were not detected. Carbapenemase-gene analysis showed that selected treated-wastewater isolates carried *bla*VIM and/or *bla*NDM. Because carbapenemase genes were detected only in treated-wastewater isolates, these findings should be interpreted cautiously and considered together with the phenotypic imipenem-resistance results.

However, after treatment, 4 of 18 *E. coli* isolates were resistant to imipenem. Genotypic analysis revealed that 2 of these 4 isolates carried bla_{NDM}, whereas all four carried bla_{VIM} (Figure 5). Because multiple isolates were recovered from the same sample, isolate-level comparisons are exploratory and should not be interpreted as confirmatory evidence of treatment-associated selection.

Table 3. Physicochemical parameters of untreated and treated wastewater.

Parameter	Untreated wastewater		Treated wastewater		p-value
	Low	High	Low	High	
pH	6.9 ± 0.04	7.5 ± 0.04	7.1 ± 0.04	7.55 ± 0.04	> 0.05
TSS (mg/L)	120.25 ± 20.23	145.86 ± 24.55	46 ± 7.74	55 ± 8.84	< 0.001
BOD_5 (20°C, mg/L)	107.23 ± 25.74	165.42 ± 39.70	20.85 ± 5.00	23.5 ± 5.64	< 0.001
COD (mg/L)	207.18 ± 25.90	258.32 ± 32.29	40.72 ± 5.09	49.56 ± 6.02	< 0.001

TSS: total suspended solids; COD: chemical oxygen demand; BOD_5: biochemical oxygen demand.

Table 4. *E. coli* strains' antibiotic resistance phenotypic.

Antibiotic type	Untreated wastewater (n=15)			Treated wastewater (n=18)		
	(S)	(I)	(R)	(S)	(I)	(R)
Ampicillin (AMP 10)	2 (13.3)	0 (0)	13 (86.7)	0 (0)	0 (0)	18 (100)
Amoxycillin/clavulanic acid (AMC 30)	11 (73.3)	0 (0)	4 (26.7)	8 (44.4)	3 (16.7)	7 (38.9)
Cefazolin (KZ 30)	1 (6.7)	6 (40)	8 (53.3)	0 (0)	1 (5.6)	17 (94.4)
Imipenem (IPM 10)	12 (80)	3 (20)	0 (0)	14 (77.8)	0 (0)	4 (22.2)
Gentamicin (GEN 10)	10 (66.7)	0 (0)	5 (33.3)	7 (38.9)	0 (0)	11 (61.1)
Tetracycline (TE 30)	2 (13.3)	1 (6.7)	12 (80)	5 (27.8)	0 (0)	13 (72.2)
Ciprofloxacin (CIP 5)	5 (33.3)	3 (20)	7 (46.7)	6 (33.3)	0 (0)	12 (66.7)
Sulfamethoxazole-trimethoprim (SXT 25)	6 (40)	1 (6.7)	8 (53.3)	7 (38.9)	2 (11.1)	9 (50)
Chloramphenicol (C 50)	7 (46.7)	0	8 (53.3)	6 (33.3)	0 (0)	12 (66.7)
Fosfomycin (FO 200)	6 (40)	1 (6.7)	8 (53.3)	17 (94.4)	0 (0)	1 (5.6)
Colistin	5 (33.3)	-	10 (66.7)	12 (66.7)	-	6 (33.3)

S: susceptible; I: intermediate; R: resistant. Values are shown as number of isolates (%). "-": not applicable for colistin interpretation. Colistin was interpreted by broth microdilution MIC testing using the EUCAST Enterobacterales breakpoint: susceptible, MIC ≤2 mg/L; resistant, MIC >2 mg/L.

Table 5. Genotypes of *E. coli* isolated with antibiotic resistance found in wastewater samples.

Sample No.	Code strain	Genotype														
		Beta-lactamase (ESBL)						Colistin					Carbapenem			
		TEM	SHV	CTX				*mcr*					OXA48	VIM	NDM	KPC
				M-1	M-2	M-9	M-8/25	1	2	3	4	5				
Untreated wastewater																
1	CE26	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	CE27	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	CE28	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
2	CE29	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	CE30	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
	CE31	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
4	CE32	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	CE33	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	CE34	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
9	CE96	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
	CE97	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	CE98	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
10	CE99	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	CE100	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-
	CE101	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treated wastewater																
1	DE26	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-
	DE27	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	DE28	+	-	+	-	-	-	-	-	-	-	-	-	+	-	-
2	DE29	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-
	DE30	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-
	DE31	+	-	-	-	-	-	-	-	-	-	-	-	+	+	-
4	DE32	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DE33	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
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5	DE35	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DE36	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
	DE37	+	-	-	-	+	-	+	-	-	-	-	-	-	-	-
6	DE38	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-
	DE39	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-
	DE40	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-
8	DE81	-	-	+	-	-	-	+	-	-	-	-	-	+	+	-
	DE82	-	-	+	-	-	-	+	-	-	-	-	-	-	-	-
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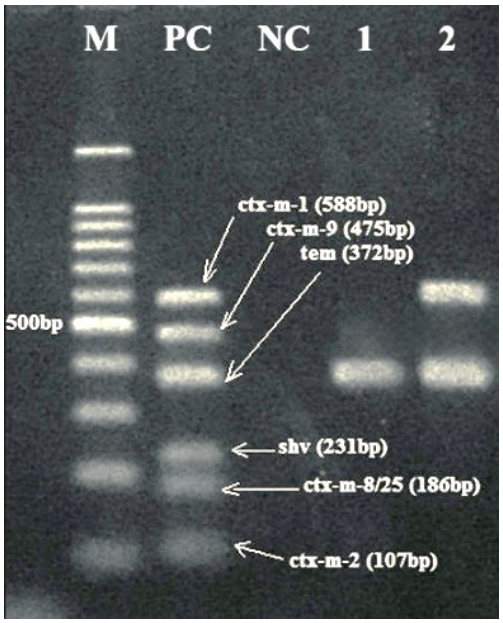

Figure 3. Results of PCR detection of ESBL. Lane (M): Ladder 100 bp; Lane PC: Positive ESBL; Lane NC: Negative; Lane 1: sample DE 31 (bla_{TEM}); Lane 2: sample DE 26 (bla_{TEM} + $bla_{CTX\text{-}M\text{-}1}$) **(Prepared by Authors, 2026)**.

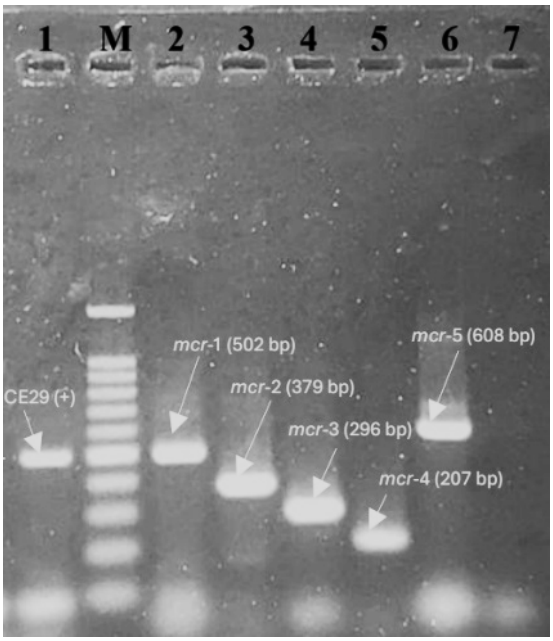

Figure 4. Results of PCR detection of *mcr*. Lane (M): ladder 100 bp; Lane 1: sample DE 26 (*mcr*-1); Lane 2: *mcr*-1 (502 bp); Lane 3: *mcr*-2 (379 bp); *mcr*-3 (296 bp); Lane 5: *mcr*-4 (207 bp); Lane 6: *mcr*-5 (608 bp); Lane 7: control negative. **(Prepared by Authors, 2026)**.

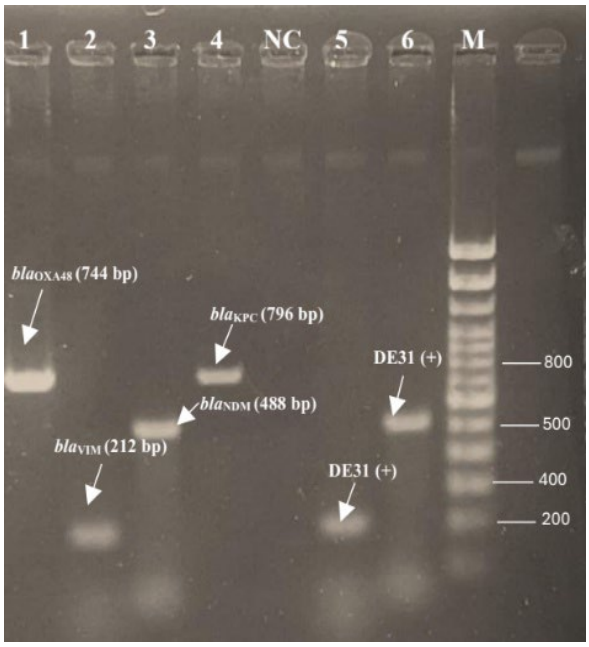

Figure 5. Results of PCR detection gene of carbapenem. Lane 1: *bla*$_{OXA48}$ (744 bp); Lane 2: *bla*$_{VIM}$ (212 bp); Lane 3: *bla*$_{NDM}$ (488 bp); Lane 4: *bla*$_{KPC}$ (796 bp); Lane NC: control negative; Lane 5: sample DE31 (positive *bla*$_{VIM}$); Lane 6: sample DE31 (positive *bla*$_{NDM}$) Lane (M): ladder 100 bp. **(Prepared by Authors, 2026)**.

4. Discussion

The physicochemical results showed that the wastewater treatment system at Sa Dec General Hospital reduced conventional pollutants and met the effluent discharge standards specified in QCVN 28:2010/BTNMT, the Vietnamese technical regulation on healthcare wastewater **(39)**. However, culture-based results showed that presumptive antibiotic-resistant *E. coli* remained detectable after treatment. This indicates that meeting conventional physicochemical discharge standards does not necessarily ensure complete removal of antimicrobial-resistant bacteria or resistance genes.

The persistence of resistant *E. coli* after chlorination is consistent with previous reports. Huang et al **(40)** found that chlorination of secondary effluent did not completely eradicate antibiotic-resistant *E. coli* and reported a higher proportion of chloramphenicol-resistant isolates after treatment. In the present study, chloramphenicol resistance was also observed more frequently among treated-wastewater isolates than untreated-wastewater isolates. However, because multiple isolates were recovered from some samples and the isolate-level comparisons were exploratory, this difference should be interpreted cautiously. Other studies have also reported persistence of antibiotic-resistant bacteria after conventional wastewater treatment or disinfection, including chlorine and ultraviolet treatment **(30, 41, 42)**.

High levels of antibiotic resistance were observed among isolates recovered from treated wastewater. All treated-wastewater isolates were resistant to ampicillin, which is consistent with reports from hospital wastewater in Ghana and Central Europe, where high or complete resistance to ampicillin among *E. coli* isolates has also been described **(43, 44)**. The high frequency of ampicillin resistance is not unexpected, given the widespread use of β-lactam antibiotics and the common occurrence of β-lactamase-mediated resistance in environmental and clinical *E. coli* populations. These findings support the role of hospital wastewater as a potential reservoir of antibiotic-resistant bacteria.

The detection of *bla*TEM and *bla*CTX-M group genes in wastewater isolates is also consistent with previous studies from Viet Nam. Lien et al **(45)** reported high rates of ESBL-producing *E. coli* and frequent detection of *bla*TEM and *bla*CTX-M genes in hospital wastewater samples from Hanoi. Similar resistance genes have also been reported in aquatic environments in Viet Nam **(46, 47)**. In the present study, *bla*TEM, *bla*CTX-M-1 group, and *bla*CTX-M-9 group genes were detected in isolates from both untreated and treated wastewater, suggesting that these resistance determinants can remain detectable after routine treatment. The absence of *bla*SHV in the tested isolates is also plausible, as SHV-type β-lactamases are more commonly associated with *Klebsiella* spp. than with *E. coli* **(48)**.

The detection of *mcr-1* in both untreated and treated wastewater isolates is of particular concern. The *mcr-1* gene is plasmid-mediated and can

contribute to transferable colistin resistance. Because colistin is often considered a last-resort antibiotic for severe infections caused by multidrug-resistant Gram-negative bacteria, the environmental presence of *mcr*-positive *E. coli* deserves attention **(23)**. Although fewer treated-wastewater isolates carried *mcr-1* than untreated-wastewater isolates in this study, the persistence of *mcr-1*-positive isolates after treatment suggests that routine chlorination may not fully remove this resistance determinant under the conditions evaluated.

Carbapenemase genes were detected in selected treated-wastewater isolates, including *bla*VIM and *bla*NDM. These genes are clinically important because they are often associated with mobile genetic elements and may spread between bacterial populations **(49)**. However, the present study did not investigate plasmid transfer, gene expression, strain relatedness, or whole-genome characteristics. Therefore, the detection of carbapenemase genes after treatment should be interpreted as evidence of persistence or occurrence, not as proof that chlorination selected for carbapenem-resistant strains. Previous studies have suggested that sublethal disinfectant exposure may influence horizontal gene transfer or release extracellular DNA from damaged cells **(50)**, but this mechanism was not directly tested in the present work. Further studies using longitudinal sampling, quantitative PCR, culture-based enumeration, and whole-genome sequencing are needed to clarify whether post-treatment detection reflects survival, regrowth, selection, or sampling variation.

Overall, the findings suggest that conventional hospital wastewater treatment with chlorination may reduce physicochemical pollution while still allowing detectable antibiotic-resistant *E. coli* and selected resistance genes to remain in treated effluent. This highlights the importance of routine microbiological and molecular monitoring of hospital wastewater, especially in settings where treated effluent is released into the surrounding environment.

This study has several limitations. First, the sample size was relatively small, and samples were collected from a single hospital, which limits generalizability. Second, multiple isolates were recovered from some wastewater samples; therefore, isolate-level resistance comparisons were exploratory and should not be considered fully independent. Third, colony counts and concentration-based removal efficiencies were not reported, so the study could not quantify bacterial-load reduction. Fourth, conventional PCR detected only the presence or absence of selected resistance genes and did not provide quantitative gene abundance. Finally, strain typing, metagenomic analysis, and whole-genome sequencing were not performed, limiting interpretation of isolate relatedness, broader resistome composition, and possible transmission pathways.

5. Conclusion

This study showed that the wastewater treatment system at Sa Dec General Hospital reduced conventional physicochemical pollutants to meet discharge standards, but did not completely eliminate presumptive antibiotic-resistant *E. coli* or selected resistance genes. High levels of resistance, particularly to ampicillin, were observed among isolates recovered after chlorination, and clinically important resistance genes, including *bla*TEM, *bla*CTX-M group genes, *mcr-1*, *bla*NDM, and *bla*VIM, were detected in wastewater isolates. These findings suggest that chlorination at 25 mg/L may be insufficient to fully control antibiotic-resistant *E. coli* under the conditions evaluated in this hospital wastewater system. Routine monitoring of antimicrobial resistance in hospital wastewater, together with optimized disinfection or complementary treatment strategies, may help reduce environmental dissemination risks. Future studies should include larger sample sizes, multiple hospitals, quantitative gene analysis, and whole-genome sequencing to better characterize resistant isolates and their potential transmission pathways.

6. Declarations

6.1 Acknowledgment

The authors thank Sa Dec General Hospital for permission to collect wastewater samples and the staff of the Institute of Food and Biotechnology, Can Tho University, for technical assistance during sample processing and analysis.

6.2 Ethical Considerations

This is an observational study. The Institute of Food and Biotechnology, Can Tho University, Can Tho City, Viet Nam has confirmed that no ethical approval is required.

6.3 Authors' Contributions

Phong Thanh Ngo, Phong Xuan Huynh and Hung Do Tran contributed to the study design and laboratory experiments. Ho Long Phan and Chinh Van Dang performed data analysis. Tri Trung Le performed sample collection and sample coding. All authors read and approved the final version.

6.4 Conflict of Interests

The authors declare no conflict of interest.

6.5 Financial Support and Sponsorship

The authors declare that no funds, grants, or other support were received during the preparation of this manuscript.

6.6 Using Artificial Intelligence Tools (AI Tools)

Artificial intelligence tools were used to assist with language editing, grammar correction, and improvement of manuscript clarity. The authors reviewed, revised, and approved all AI-assisted changes and take full responsibility for the accuracy, integrity, and final content of the manuscript.

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