Journal of Current Science and Technology, July - September 2025 Vol. 15 No. 3, Article 121

ISSN 2630-0656 (Online)

Cite this article: Meeprawat, S., Jaikaew, P., Chanklan, R., Pannanusorn, S., & Utaida, S. (2025). Environmental surveillance of gram-negative bacteria and *bla* genes in hospital facilities and surrounding waters in Thailand. *Journal of Current Science and Technology*, *15*(3), Article 121. https://doi.org/10.59796/jcst.V15N3.2025.121

Journal of Current Science and Technology

Journal homepage: https://jcst.rsu.ac.th

Environmental Surveillance of Gram-Negative Bacteria and *bla* Genes in Hospital Facilities and Surrounding Waters in Thailand

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Received 17 January 2025; Revised 7 April 2025; Accepted 11 April 2025; Published online 15 June 2025

Abstract

To reveal the real time prevalent situation of antibiotic-resistant bacteria (ARB) and *bla* genes in Thailand, we monitored 83 isolates of Gram-negative bacteria (GNB) from hospital facilities and surrounding environmental waters. 16*S* rRNA gene sequencing was performed. Polymerase chain reactions were employed for *bla* gene detection. Disk diffusion was used for antimicrobial susceptibility testing. As a result, *Enterobacter mori* (20%) and *Klebsiella pneumoniae* (17.14%) were prevalent in hospital facilities, while *K. pneumoniae* (27.08%) and *Enterobacter cloacae* (14.58%) prevailed in water samples. Ampicillin resistance rates were highest, at 65.71% and 66.67% in hospital and water isolates, respectively. *Enterobacter* species from water samples exhibited multidrug-resistant characteristics. bla_{SHV} and bla_{TEM} were highly prevalent, 91.43% and 89.58% in various bacterial species from hospital facilities and water samples, respectively. The coexistence of bla_{SHV} and bla_{TEM} and bla_{NDM} was the most common overall (16.87%). The prevalence of the same bacterial species and *bla* genes in both sectors suggests the cross-transfer of ARGs and resistant bacteria between different environments, hospital and water. The findings emphasize concerns about the safety of water sources and bacterial contamination in hospital facilities.

Keywords: *dissemination; Gram-negative bacteria; bla gene; hospital facilities; water environment; ARB; ARGs*

1. Introduction

Gram-negative bacteria (GNB) are one of the major causes of healthcare-and community-acquired infections worldwide, including Thailand (Hongsuwan et al., 2014; Khoka, 2020; Lim et al., 2016; WHO, 2024). GNB resistant to antibiotics at a critical level, as reported by the World Health Organization (WHO) (2024) and the Centers for Disease Control and Prevention (CDC) (2022), include carbapenem-resistant *Enterobacterales* (CRE) and ESBL-producing *Enterobacterales* (E-ESBL). Reports of members of this group, such as *Enterobacter cloacae a*nd *Klebsiella pneumoniae*, being resistant to beta-lactam antibiotics have significantly increased in Thailand from 2000 to 2020 (Department of Disease Control, 2022). The problem of antibiotic resistance in GNB renders medications ineffective and facilitates the spread of these resistant bacteria into the environment. Epidemiological studies on environmental bacterial transmission show that water environments can harbor and disseminate many clinically important bacteria. The presence of antibiotic-resistant bacteria

(ARB) has been detected in many water sources around the world, especially in low- and middle-income countries (Ahmad et al., 2021; Andrade et al., 2020; Falgenhauer et al., 2019; Solaiman et al., 2022). Concerningly, the presence of ARB in water can contribute to the evolution of resistance among bacterial communities in aquatic habitats, contaminating water used for various purposes (Ahmad et al., 2021). This emphasizes a crucial aspect of antibiotic resistance within the framework of the One Health approach (Aslam et al., 2021; Martak et al., 2024).

Additionally, hospital facilities have been reported as dissemination ground for clinically important bacteria with the potential to spread to patients (Zahornacký et al., 2022). Recently, contamination by antibiotic-resistant bacteria on material surfaces in hospitals has been reported, particularly in oncology units, especially in hand sinks (Joachim et al., 2023). Furthermore, elevated levels of multidrug-resistant bacteria were found around bed rails, sinks, and computer equipment (Chansareewittaya, & Krajangcharoensakul, 2022; Odoyo et al., 2023), as well as on common inanimate surfaces in hospitals (Zahornacký et al., 2022). The discovery of ARB is not confined to healthcare facilities alone. Bacteria carrying antibiotic resistance genes (ARGs) have also been identified in various environments. Beta-lactamases (*bla*) are the largest class of ARGs and contribute to most incidences of antibiotic resistance. The *bla* genes are prevalent in various environmental waters across different regions of the world including Thailand (Ahmad et al., 2021; Assawatheptawee et al., 2017; Dawangpa et al., 2021; Miao et al., 2022; Pornmee et al., 2015; Solaiman et al., 2022).

In the past 10 years, no studies have investigated the dissemination of ARB in environmental water surrounding hospitals in our study area. Additionally, trends in antibiotic resistance among bacterial populations in both hospital environments and nearby water sources remain unknown. Moreover, there is a notable lack of information on patients with antibiotic-resistant infections, including details on the spread of ARB within the community and the environment. Beta-lactams such as aminopenicillins (Siltrakool et al., 2021), cephalosporins, and carbapenems (Anugulruengkitt et al., 2023) have been reported as the most commonly used antibiotics in several hospitals throughout Thailand. Thus, in this study, we investigated the prevalence of GNB and *bla* genes from hospital facilities and the surrounding environmental waters. We also determined the antibiotic resistance of the isolated bacteria against different groups of beta-lactam antibiotics. We then analyzed the relevance of the resistance phenotype and detected ARGs.

2. Objectives

To investigate the real time prevalence and dissemination of Gram-negative bacteria and *bl*a genes in hospital facilities and surrounding environmental water sources at Thammasat University, Rangsit Center, Thailand, and to evaluate antibiotic resistance patterns and gene associations.

3. Materials and Methods

3.1 Environmental Sampling

Municipal surface water samples were collected from fifteen locations across different areas within Thammasat University, Rangsit Center, Thailand. These were near the school building (spots 1-3), in the canteen (spots 4-6), the field and sports building (spots 7-9), the dormitories (spots 10-12), and other multipurpose buildings (spots 13-15) (Figure 1). Some water sites were connected to others through water routes within the university (Figure 1). Sampling was conducted between 9:00 a.m. and 12:00 noon from November 2021 to January 2022. The samples were collected at a depth of approximately 30 cm below the surface using sterile glass bottles. A minimum of 100 mL of water was collected. The sample bottles were kept in a foam box to maintain a temperature range of 4-10 °C during transportation to the laboratory (Adamu et al., 2018; Bhumbla et al., 2020). All water samples were processed within 8 hours after the collection.

For hospital facilities, inanimate high-frequency hand-touch surfaces were randomly sampled from fourteen locations in six different buildings (Dulasophak; Mr. Suwaphan Sanitwong, Kittiwattana, Panja Sayalak, Thai Military Bank, and the Canteen) at Thammasat University Hospital (Figure 2). These included 2 canteen tables, 5 main entrance doors, 3 hospital waiting chairs, 3 corridor handrails, and an escalator handrail. Sampling was conducted between 11:00 a.m. and 1:00 p.m. from November 2021 to February 2022. Sterile cotton swabs soaked in 0.85% (w/v) NaCl solution were used to collect samples and then stored in sterile tubes containing 10 mL of 0.85% (w/v) NaCl before transportation to the laboratory for cultivation (Lemmen et al., 2004; Wang et al., 2017).

3.2 Bacterial Cultivation

All samples were cultured in Luria-Bertani (LB; BD) broth at 37 °C with shaking at 200 rpm for 24 h. Specifically, 5 mL of each water sample was used. Forty mL of LB broth was employed for each culture. Afterward, the liquid cultures were then cross streaked onto MacConkey (MC; HIMEDIA, Mumbai, India) agar and incubated at 37 °C for 24 h. Distinct single colonies that grew on MC agar were further subcultured on LB agar to obtain pure cultures. Bacterial colonies were subsequently cultured in LB broth at 37 °C in a shaker at 200 rpm for 24 h before being stored in LB broth containing 30% (v/v) glycerol at -20 °C.

3.3 Bacterial Identification and Phylogenetic Tree Construction

Gram staining and basic biochemical tests, including carbohydrate fermentation; indole synthesis; methyl red; Voges-Proskauer; hydrogen sulfide formation; urease synthesis; citrate utilization; and nitrate reduction, were conducted to identify bacterial isolates (Bergey, & Holt, 1993; Cappuccino, & Welsh, 2018). Identification was confirmed by 16*S* rRNA gene sequencing. Genomic DNA was extracted using GF-1 Bacterial DNA Extraction Kit (Vivantis, Malaysia). Specific primers for amplifying the full length of the 16*S* rRNA gene, spanning 1500 base pairs, were designed (Table 1) based on the consensus sequence using the Primer-Blast program available in the National Center for Biotechnology Information (NCBI) database. PCR products were purified using GF-1 PCR Clean-up Kit (Vivantis, Malaysia) before being subjected to Sanger sequencing method provided by Macrogen (Seoul, South Korea). The resulting sequences were compared with reference sequences available in GenBank by employing the BLASTn (Basic Local Alignment Search Tool) program provided by NCBI. Multiple sequence alignments were performed using the ClustalW algorithm in the MEGA X program. A UPGMA (Sneath, & Sokal, 1973) phylogenetic tree was constructed based on the Maximum Composite Likelihood model (Tamura et al., 2004) and the bootstrap method (1000 replications). Reference sequences of the 16*S* rRNA gene from Gram-negative bacteria were obtained from the GenBank database (CP128719.1, CP034136.1, CP126341.1, OW969784.1, CP039303.1, CP050447.1, CP099014.1, CP076369.1, AM231709.1, CP031910.1, NR_184601.1, OQ689074.1, AB999815.1, NR_137380.1, MT311965.1, and MN094126.1). Deviations from neutral evolution or nucleotide variation were assessed using the Tajima neutrality test (Tajima, 1989).

3.4 Antimicrobial Susceptibility Test

Antibiotic susceptibility test was performed using the Kirby-Bauer disk diffusion assay, following the standardized protocol outlined by the Clinical and Laboratory Standards Institute (CLSI) M02-A11 (CLSI, 2012). Each bacterial isolate was tested against individual antibiotics in triplicate. The diameter of the inhibition zones around each antibiotic disc was measured in millimeters and subsequently compared to the zone diameter values recommended by CLSI M100 (CLSI, 2020). Accordingly, the results were reported as susceptible (S) or intermediate (I) or resistant (R). *Escherichia coli* ATCC 25922 was used as a control strain. The following antibiotics and their respective disk concentrations were employed: ampicillin: AMP (10 µg), amoxicillin/clavulanic acid: AMC (20/10 µg), cefuroxime: CXM (30 µg), cefotaxime: CTX (30 µg), ceftriaxone: CRO (30 µg), ceftazidime: CAZ (30 µg), meropenem: MEM (10 µg), imipenem: IPM (10 µg), doripenem: DOR (10 µg), and aztreonam: ATM (30 µg).

3.5 Antibiotic Resistance Genes Detection

Bacterial cell lysates were prepared using the following method. Cell pellets from overnight cultures in LB were collected, washed once and twice with 500 µL of 0.5x TAE buffer (Sambrook, & Russell, 2001) and lysis buffer (Utaida et al., 2003) solutions, respectively at 10,000 rpm for 5 min before being resuspended in 100 µL of TE buffer solution, pH 8.0 (Sambrook, & Russell, 2001). The boiling of the cell suspension was then performed in a water bath for 10 min, the supernatant was collected and stored at -20 °C before being used as DNA template (Cheng et al., 2008) within 2 weeks. PCR was performed employing *Taq* DNA polymerase (Vivantis, Malaysia) and specific primers listed in Table 1. PCR reactions for bla_{TEM} and $bla_{\text{OXA-58}}$ were 5 min of initial denaturation at 94.0 °C, followed by 35 cycles at 94.0°C for 40 s, 59.0 °C for 1 min, and 72.0 °C for 45 s, and a final elongation at 72.0 °C for 5 min. A similar PCR reaction was used for bla_{SHV}, $bla_{\text{CTX-M}}$, bla_{NDM}, and $bla_{\text{OXA-24}}$, except that the annealing temperatures were 61.0, 50.0, 52.0, and 62.0 °C for 1 min, respectively. The bla_{AmpC} and $bla_{\text{OXA-48}}$ PCR reactions were performed using the following protocol: an initial denaturation at 94 °C for 5 min, followed by 30 cycles at 94 °C for 40 s, 51°C for 45 s, and 72 °C for 45 s, and a final extension at 72 °C for 7 min. PCR

products were analyzed using 1.7% (w/v) agarose gel electrophoresis.

3.6 Diversity Indexes

The diversity indexes (Hammond, & Pokorný, 2020; Issa, 2019; Strong, 2016) were calculated as follows:

Shannon-Wiener index *(H),* $= -\sum_{i=1}^{s} pi*$ ln(pi); where pi = is the abundance of the i th species, and i is one through S, S = species richness

Evenness (J) = H/ln$S$

Sørensen's similarity coefficient (S_s) = 2A/(2A+B+C); where A = the total number of species in both communities, B and C = the total number of unique species in each community

4. Results and Discussion

4.1 The Dissemination of GNB

In this study, a total of 366 (203 and 163 from water and hospital facilities, respectively) different single colonies obtained from primary culture using MC agar were selected for subculturing. Only 224 cultures, of which 115 and 109 cultures were from water and hospital facilities, respectively, were recovered for further biochemical tests. Representative isolates with similar biochemical profiles were then confirmed by 16*S* rRNA gene sequencing. Eventually, a total of 83 isolates of GNB (Table 2 and Table 3) were obtained. Of these, 35 isolates were from inanimate, high-frequency hand-touched material surface areas within Thammasat University Hospital, while the remaining 48 isolates were from the surfaces of surrounding environmental water sources. The three most predominant species found on material surfaces were *Enterobacter mori* (20%), followed by *Klebsiella pneumoniae* (17.14%) and *E. cloacae* (8.57%), respectively. Similarly, *K. pneumoniae*, 27.08%, followed by *E. cloacae*, 14.58%, and *Escherichia coli*, 12.50%, were the three most prevalent in water samples as shown in Table 4. Consistently, existing studies reported the occurrence of these bacteria in hospital facilities and environmental water in various places, including Thailand (Chansareewittaya, & Krajangcharoensakul, 2022; Kiddee et al., 2018; Kotzamanidis et al., 2024; Yuan, & Pian, 2023). The prevalence of such bacteria in hospitals might be related to colonization on surfaces in healthcare facilities, which can be influenced by various factors, including the bacteria's ability to form biofilms, allowing them to remain on moist hospital surfaces for the long term (Bonadonna et al., 2017; Cruz-Córdova et al., 2014). Hospital environmental factors such as temperature and humidity can affect the concentration of bacteria found in healthcare facilities (Onmek et al., 2020). Additionally, the prevalence of *K. pneumoniae* across various water sources found in this study may be attributed to environmental factors that facilitate bacterial growth, particularly during seasonal periods such as winter, which tend to be humid and may be influenced by the preceding rainy season when the samples were collected. The rain that fell washed soil or remains of plants and animals, which harbor bacteria, into water sources, thereby promoting the prevalence of bacteria. Ruangsombat et al., (2024) reported that the higher the amount of rainfall, the greater the bacterial contamination in the water. Additionally, canals in the university, which were our sampling sites, received the effluent from wastewater treatment plants from both the university hospital and the central plants. These sites are connected to others through water routes as shown in Figure 1. Furthermore, our water sampling sites were close to the sites with high human activities such as the water sources near dormitories and canteens. Therefore, there is a possibility that bacterial contamination from both hospital and daily activities may enter the water sources in that area and spread to other areas. Additionally, inappropriate handling of wastewater from various activities such as direct dumping into nearby water sources without proper treatment, is considered one of the reasons why these bacteria spread more in water sources.

We found similar bacterial species between the two sectors studied. Since our research was designed as an observation study, the number of isolates sequenced, therefore, was too small to perform statistical analyses. Thus, observations were supported using diversity indexes. No difference in species diversity was observed, as indicated by species richness values of 19 and 18 for surface water and hospital material surfaces, respectively. Similarly, the Shannon-Wiener diversity indexes were close for both hospital and water environments, 2.61 and 2.47, respectively. The evenness indexes were similar, 0.90 and 0.84 for material surfaces and water sources, respectively (Table 5). Diversity indexes depend on both species richness and evenness within a community (Wu et al., 2017). Additionally, Sørensen's similarity coefficient value of 0.38 revealed some similarity of bacterial species between the two sectors (Table 5).

The randomly selected sampling sites were, in theory, not far from each other. The same bacterial species were found at similar frequency in both water sources (outside the hospital area) and hospital

material surfaces, suggesting possible bacterial dissemination between these areas. Nevertheless, it is possible that the observed similarity in bacterial isolates may be due to the limited number of isolates analyzed (83 from 29 sampling sites). Sampling was conducted between November 2021 and February 2022, during the COVID-19 pandemic. Thailand, like many countries worldwide, was affected. Several public health strategies were implemented to control the spread of the disease. The online classroom system was adopted for Thammasat University, Rangsit Center. Access to various locations was limited. This posed a major limitation for sampling outside the university. Therefore, sampling efforts were focused on local areas within the university.

Figure 1 Map of water sampling sites within Thammasat University, Rangsit Center. The 15 numbered spots correspond to: school buildings (1–3), canteens (4–6), field and sports buildings (7–9), dormitories (10–12), and other multipurpose buildings (13–15). Blue lines indicate water routes. Adapted from the Thammasat Project Smart City (2017)

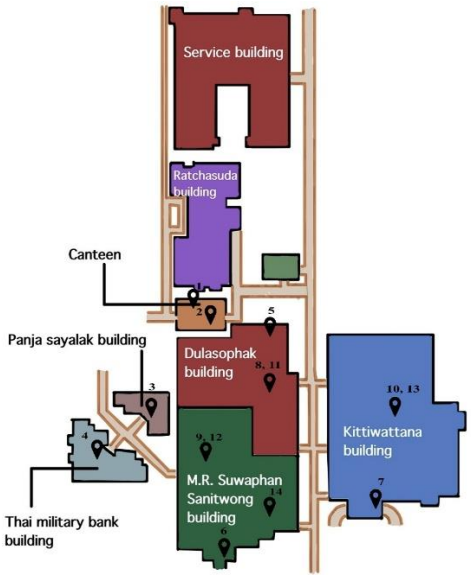

Figure 2 Sampling sites within Thammasat University Hospital, indicating 14 designated locations. Map data obtained from the Building Department of Thammasat University Hospital (Thammasat Project Smart City, 2017)

Table 1 Primers used in this study

Molecular Class	Target gene	Sequence 5'- 3'	Length	Tm (°C)	Product size (bp)	References
	16*S* rRNA	f- TGT GTG GGC ACT CGA AGA r- CAG ACA ATC TGT GTG GGC AC	18 20	61.8 61.2	1500-2000	This study
A	*bla*$_{TEM}$	*bla*$_{TEM}$-f TTT CGT GTC GCC CTT ATT CC	20	61.1	404	Hassan et al., 2013; Kasemsan et al., 2015
		bla$_{TEM}$-r ATC GTT GTC AGA AGT AAG TTG G	22	56.5		
	bla$_{SHV}$	*bla*$_{SHV}$-f CGC CTG TGT ATT ATC TCC CT	20	58.3	294	Hassan et al., 2013; Kasemsan et al., 2015
		bla$_{SHV}$-r CGA GTA GTC CAC CAG ATC CT	20	61.3		
B	*bla*$_{NDM}$	*bla*$_{NDM}$-f GGT TTG GCG ATC TGG TTT TC	20	59.6	621	Joshi et al., 2017; Poirel et al., 2011; Preechachuawong et al., 2015
		bla$_{NDM}$-r CGG AAT GGC TCA TCA CGA TC	20	60.9		
C	*bla*$_{AmpC}$	*bla*$_{AmpC}$-f ATC AAA ACT GGC AGC CG	17	57.7	701	Akpaka et al., 2021; Messai et al., 2006
		bla$_{AmpC}$-r GAG CCC GTT TTA TGC ACC CA	20	63		
D	*bla*$_{OXA24}$	*bla*$_{OXA-24}$-f GGT TAG TTG GCC CCC TTA AA	20	60.7	246	Joshi et al., 2017; Woodford et al., 2006
		bla$_{OXA-24}$-r AGT TGA GCG AAA AGG GGA TT	20	59.1		
	bla$_{OXA-58}$	*bla*$_{OXA-58}$-f AAG TAT TGG GGC TTG TGC TG	20	59.7	599	Joshi et al., 2017; Woodford et al., 2006
		bla$_{OXA-58}$-r CCC CTC TGC GCT CTA CAT AC	20	62.2		
	bla$_{OXA-48}$	*bla*$_{OXA-48}$-f GCG TGG TTA AGG ATG AAC AC	20	50	438	Poirel et al., 2011; Preechachuawong et al., 2015
		bla$_{OXA-48}$-r CAT CAA GTT CAA CCC AAC CG	20	50		

Table 2 GNB isolated from environmental water

Sampling site	GPS		Date	Time	Number
	Longitude: E	Latitude: N			
1. Faculty of Medicine and Pharmacy building	67.4321	155.6318	7.11.2021	9.48 a.m.	5 isolates
2. Faculty of Science and Technology building, lecture classroom 5 (LC.5)	67.3597	155.6585	7.11.2021	10.14 a.m.	4 isolates
3. Faculty of Social Sciences building (SC. 1)	67.2946	155.6035	7.11.2021	11.11 a.m.	4 isolates
4. Science Canteen	67.3689	155.6307	7.11.2021	10.07 a.m.	4 isolates
5. Green Canteen	67.2906	155.6434	19.12.2021	10.21 a.m.	2 isolates
6. Social Science and Engineering Canteen	67.3247	155.6076	19.12.2021	11.22 a.m.	3 isolates
7. Futsal Stadium at Faculty of Engineering	67.3725	155.6081	2.1.2022	10.39 a.m.	3 isolates
8. Thammasat Water Sport Center	67.2887	155.5615	19.12.2021	11.02 a.m.	3 isolates
9. Main Stadium	67.2474	155.5817	19.12.2021	10.48 a.m.	3 isolates
10. F and M zone: dormitory	67.2695	155.6400	2.1.2022	11.08 a.m.	1 isolate
11. C zone: dormitory	67.2519	155.6510	19.12.2021	10.34 a.m.	2 isolates
12. B zone: dormitory	67.2336	155.7056	16.1.2022	11.49 a.m.	3 isolates
13. Puey Park for People and Sustainability	67.3976	155.6284	2.1.2022	10.15 a.m.	5 isolates
14. Krom Luang Naradhiwas Rajanagarinda Learning Centre	67.3019	155.6270	2.1.2022	10.55 a.m.	3 isolates
15. Puey Ungphakorn Library	67.2943	155.6129	2/1/2565	11.02 a.m.	3 isolates
Total					**48 isolates**

Table 3 GNB isolated from hospital material surfaces

Sampling site	Date	Time	Number
1. Canteen table 1	27.01.2022	12.51 p.m.	4 isolates
2. Canteen table 2	27.01.2022	13.09 p.m.	2 isolates
3. The main entrance door of Panja Sayalak building (inpatient wards)	2.2.2022	11.49 a.m.	2 isolates
4. The main entrance door of Thai Military Bank building (inpatient wards and rehabilitation center for stroke patients)	27.01.2022	11.52 a.m.	3 isolates
5. The main entrance door of Dulasophak building (outpatient examination unit, pediatrics, etc.)	27.01.2022	12.49 p.m.	3 isolates
6. The main entrance door of Mr. Suwaphan Sanitwong building (outpatient wards)	27.01.2022	12.36 p.m.	1 isolate
7. The main entrance door of Kittiwattana building (emergency unit)	27.01.2022	12.32 p.m.	3 isolates
8. Hospital waiting chair of Dulasophak building	27.01.2022	11.58 a.m.	2 isolates
9. Hospital waiting chair of Mr. Suwaphan Sanitwong building	27.01.2022	12.13 p.m.	2 isolates
10. Hospital waiting chair of Kittiwattana building	27.01.2022	12.07 p.m.	3 isolates
11. Corridor handrail of Dulasophak building	2.2.2022	12.58 p.m.	3 isolates
12. Corridor handrail of Mr. Suwaphan Sanitwong building	2.2.2022	11.50 a.m.	2 isolates
13. Corridor handrail of Kittiwattana building	2.2.2022	12.00 p.m.	2 isolates
14. Escalator handrail of Mr. Suwaphan Sanitwong building	27.01.2022	12.14 p.m.	3 isolates
Total			**35 isolates**

Table 4 GNB (percentages) isolated from environmental water and hospital material surfaces in this study

Bacteria	Sample sources		
	Total (N=83)	Environmental water (N=48)	Hospital material surfaces (N=35)
Atlantibacter hermannii	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Citrobacter koseri	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Citrobacter tructae	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Enterobacter asburiae	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Enterobacter bugandensis	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Enterobacter cloacae	15.63 (10/83)	14.58 (7/48)	8.57 (3/35)
Enterobacter mori	14.06 (9/83)	4.17 (2/48)	20.00 (7/35)
Enterobacter quasihormaechei	3.13 (2/83)	0.00 (0/48)	5.71 (2/35)
Enterobacter quasiroggenkampii	3.13 (2/83)	4.17 (2/48)	0.00 (0/35)
Enterobacter sichuanensis	4.69 (3/83)	2.08 (1/48)	5.71 (2/35)
Enterobacter wuhouensis	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Escherichia coli	9.38 (6/83)	12.50 (6/48)	0.00 (0/35)
Escherichia fergusonii	3.13 (2/83)	2.08 (1/48)	2.86 (1/35)
Franconibacter helveticus	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Franconibacter pulveris	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Huaxiibacter chinensis	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Klebsiella aerogenes	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Klebsiella oxytoca	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Klebsiella pneumoniae	29.69 (20/83)	27.08 (13/48)	17.14 (6/35)
Klebsiella quasipneumoniae	9.38 (6/83)	8.33 (4/48)	5.17 (2/35)
Klebsiella variicola	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Mixta intestinalis	3.13 (2/83)	0.00 (0/48)	5.71 (2/35)
Pantoea agglomerans	4.69 (3/83)	4.17 (2/48)	2.86 (1/35)
Pantoea dispersa	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Proteus mirabilis	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Pseudocitrobacter faecalis	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Pseudocitrobacter vendiensis	1.56 (1/83)	0.00 (0/48)	2.86 (1/35)
Serratia marcescens	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)
Shigella dysenteriae	1.56 (1/83)	2.08 (1/48)	0.00 (0/35)

Table 5 Diversity indexes of GNB from environmental water and hospital material surfaces

Index	Environmental water	Hospital material surfaces
Species richness	19	18
Shannon-Wiener diversity index	2.47	2.61
Evenness	0.84	0.90
SØrensen's similarity coefficient	0.38	

4.2 Phylogenetic Tree Analysis

The phylogenetic tree revealed that bacterial isolates in this study were grouped into two main clades, G1 and G2, based on bootstrap clustering percentages exceeding 50% from 1,000 replicates, as illustrated in Figure 3. Of the 83 isolates, 75.9% (63/83) were assigned to group G1, with a 100% bootstrap support rate. Among these, 79.16% (38/48) of water isolates and 71.4% (25/35) of hospital isolates were in group G1. The remaining 24.09% (20/83) were classified as group G2, also supported by 100% bootstrap replication. Within this group, 20.83% (10/48) were from water sources, and 28.6% (10/35) were from material surfaces. Group G1 was subdivided into clades E1, K1, K2, and C1, while G2 consisted of clade U1. Overall, most of GNB isolates shared a close evolutionary relationship, exhibiting approximately 19% nucleotide variation in the 16*S* rRNA gene sequence. The variation suggests genetic divergence within the 16*S* rRNA gene among the bacterial isolates. This variation may result from evolutionary processes and adaptive responses to environmental changes. Moreover, genetic diversity is essential for the long-term survival of bacterial strains

in diverse environments. Importantly, the phylogenetic tree shows that all clades include isolates from both hospital and water sources, suggesting that these genera can inhabit or thrive in both environments. This highlights the potential bidirectional transmission of GNB between clinical settings and environmental water sources.

4.3 Antibiotic Susceptibility Test

GNB from both hospital material surfaces and water sources exhibited the highest resistance rates to AMP: 65.71% and 66.67%, respectively. Lower resistance rates to AMC were observed: 34.29% in hospital isolates and 31.25% in water isolates (Table 6). The high resistance rates against AMP were observed for three hospital isolates, *Pseudocitrobacter vendiensis, Klebsiella,* and *Enterobacter* species (100%, 75%, and 66.67%, respectively) (Figure 4). For the water isolates, *Shigella dysenteriae*, *Citrobacter koseri*, and *Serratia marcescens* had the highest AMP resistance rate (100%), followed by 85% in *Klebsiella* spp. Similarly, the AMC resistance rate was as high as 100% in *C. koseri*, with 64.29% found in *Enterobacter* spp. (Figure 5). Consistently, high frequencies of AMP and AMC resistance have been reported in *Enterobacterales* isolated from hospital facilities and environmental water sources globally (Assawatheptawee et al., 2022; Kotzamanidis et al., 2024; Zorgani et al., 2015).

Interestingly, water isolates exhibited greater diversity in resistance profiles compared to hospital isolates (Table 7). CRO and CXM resistance were detected only in water isolates and were primarily associated with *C. koseri*. Nevertheless, intermediate levels against cephalosporins were detected in hospital isolates. Furthermore, multiple-antibiotic resistance patterns were majorly found in water isolates. A seven-antibiotic resistance pattern (AMP+AMC+CAZ+CTX+CRO+CXM+ATM) was observed in 4.17% of the water isolates, specifically *E. cloacae* isolate 10.2 and 12.1, (Table 7 and Figure 6). This is quite worrisome because previous reports have noted antibiotic adaptation and opportunistic pathogenic behavior in *Enterobacter* spp. (Ganbold et al., 2023). These bacteria can acquire mobile genetic elements leading to multidrug resistance that may be transferred to other species (Davin-Regli, & Pagès, 2015). Importantly, the presence of ARB in surface water emphasizes the role of aquatic environments as a reservoir of resistant bacteria that could be disseminated to other systems.

4.4 ARGs Profiles

The most prevalence of ARGs detected from hospital isolates were bla_{SHV} (91.43%) and bla_{TEM} (60.0%). Similarly, GNB from water samples showed the occurrence of bla_{TEM}, bla_{SHV}, and bla_{NDM}, at 89.58%, 75.0%, 52.08%, respectively (Table 8). Mostly, ARGs were detected in water isolates, especially bla_{TEM} and bla_{NDM}. *Klebsiella* and *Enterobacter* species found were highly positive for bla_{SHV}. These two bacterial species were the most prevalent in this study. Additionally, the highest prevalence of bla_{SHV} and bla_{TEM} observed is consistent with high ampicillin resistance rates observed (Table 6). It is speculated that the frequency of bla_{SHV} in hospital isolates may promote the fitness of the bacteria in inappropriate conditions such as a poor nutrition environment of the material surfaces.

The prevalence of bla_{TEM} and bla_{SHV} in various isolates from both sectors (Figure 6) were consistent with other studies. bla_{TEM} was predominantly detected in the final wastewater outlets of hospitals (Pornmee et al., 2015) and water sources in animal farms (Dawangpa et al., 2021). Ruekit et al., (2022) reported the prevalence of bla_{TEM} and bla_{SHV} among multidrug-resistant ESKAPEE bacteria in Chonburi province. Hussain (2021) indicated that these genes are often isolated from hospitals and clinics. A meta-analysis by Zhang et al., (2020) revealed the highest relative abundance of bla_{SHV} in hospital wastewater. In addition, bla_{TEM} was prevalent in river waters in Greece (Kotzamanidis et al., 2024). Devarajan et al. (2016) reported the high prevalence of bla_{NDM} and bla_{SHV} in river sediments. The presence of the same ARGs in both surface water and hospital material surfaces suggests the cross-transfer of the genes between the two sectors. The presence of ARGs in environmental water is of great concern. This could contaminate other systems through food chain causing severe impact on the One Health approach.

4.5 ARGs Patterns

In this study, 16.87% of bacterial isolates carried three genes, bla_{TEM} and bla_{SHV} and bla_{NDM}, simultaneously, with the most prevalence detected in water isolates (20.83%) (Table 9). In addition, 10.84% of all isolates showed the coexistence of bla_{TEM} and bla_{SHV} and 9.63% carried bla_{SHV} only. bla_{SHV} was the most prevalent, accounting for 20% of bacteria from hospital isolates. Consistently, Jadhav et al., (2023) reported the coexistence between bla_{SHV} and bla_{TEM} (68%) in *K. pneumoniae* from clinical samples in an Indian hospital. Furthermore, water isolates carried a

broader range of *bla* resistance genes compared to hospital isolates. Consistently, various *bla* families were prevalent in river water, as reported by Kotzamanidis et al., (2024). These findings suggest that surface water may serve as a reservoir of ARGs and contribute significantly to their dissemination. The prevalence of ARGs in water may be due to factors in horizontal gene transfer (HGT) that are likely to be common in aquatic environments (Berglund, 2015). HGT can be driven by three major mechanisms: conjugation, transformation, and transduction (Abe et al., 2020). The types of DNA transferred depend on the HGT mechanisms. Conjugative plasmids are transferred via conjugation, while chromosomal DNA and nonconjugative plasmids are transferred via transformation (Abe et al., 2020). Arias-Andres et al., (2018) demonstrated the horizontal transfer of conjugative plasmid, pKJK5, harboring trimethoprim resistance gene, *dfrA1*, from *E. coli* to diverse bacterial species under aquatic conditions. The authors showed that biofilm formation increased the frequency of plasmid transfer compared to planktonic cells. Additionally, HGT can also be mediated via membrane-vesicle (MV). Dorward et al., (1989) first reported MV-mediated transfer of R-plasmid carrying *bla* in *Neisseria gonorrhoeae* planktonic cells in liquid medium. Tran, & Boedicker (2017) demonstrated that MVs enable genetic exchange of planktonic cells between five Gram-negative bacterial species. The results suggested EVs as a general mechanism in exchange non-specialized genetic cargo. In this study, *K. pneumoniae*, with the highest biofilm-forming ability, was found among the bacterial isolates from water sources; this property facilitates the HGT (Michaelis, & Grohmann, 2023). It is also likely that water sources acquire ARGs from multiple origins, including hospital wastewater (HWW). Zhang et al., (2020) reported a high relative abundance of various ARGs in HWW.

4.6 Relationship between 16*S* rRNA Gene, Patterns of ARGs and the Responses to Antibiotics

A link was observed between water and hospital isolates. For example, *Klebsiella pneumoniae* isolates 3.1 and 1.1.5, and *K. quasipneumoniae* isolates 11.2 and 1.1.8. All these 4 isolates, genetically clustered within subgroup K1 (Figure 3), exhibited similar AMP response patterns and carried the same three resistance genes (bla_{TEM}, bla_{SHV}, and bla_{NDM}) simultaneously (Figure 6). Similar observations were made for *K. pneumoniae* isolates 5.3.1 and 8.1, which belong to the same subgroup U1. These were isolated from hospital material surfaces and surface water, respectively. The bacteria exhibited the same patterns of response to AMP and coexistence of the same four resistance genes (bla_{TEM}, bla_{SHV}, $bla_{\text{OXA-58}}$, and bla_{NDM}) in both isolates. These findings provide evidence of ARG cross-transfer between hospital and aquatic environments, which is central to this study. Additionally, water isolates from different sampling sites, *K. pneumoniae* isolates 8.3 (from sampling site 6) and 12.8 (from sampling site 9) in subgroup K2 exhibited similar antibiotic susceptibility profiles and carried the same resistance genes (Figure 6). This result highlights the possibility for the spread of ARGs in various aquatic environments. Furthermore, receiving water effluent from the same wastewater treatment plants of our sampling sites can contribute to these findings.

Antibiotics, resistant bacteria, and ARGs can contaminate the environment through wastewater from different sources, including hospitals, households, and livestock. The mixture of antibiotics, resistant bacteria, ARGs, and the environmental bacterial flora are created as a result. Such environments likely serve as hotspots for the development and dissemination of ARGs and ARB within microbial communities (Berglund, 2015).

The presence of same resistance genes in bacterial isolates exhibiting differing antibiotic susceptibility profiles was also observed in this study. For instance, *K. pneumoniae* isolates 8.5 and 5.3.5, both genetically related within the K2 subgroup, carried four resistance genes (bla_{TEM}, bla_{NDM}, bla_{SHV}, and $bla_{\text{OXA-58}}$) simultaneously. However, isolate 8.5 remained sensitive to CXM, whereas isolate 5.3.5 showed intermediate resistance to the same antibiotic (Figure 6). It is speculated that the 5.3.5, originating from a hospital environment, is more likely to be exposed to antibiotics commonly used or prescribed in clinical settings compared to isolate 8.5 from environmental water. Being exposed to drugs may increase the likelihood of bacterial adaptation to encountered antibiotics. Further investigation of antibiotic residues in both environments is necessary to substantiate this hypothesis. Additionally, examining the genetic relatedness of the two isolates could clarify the potential influence of genetic factors on their phenotypic differences. The inconsistency between antibiotic resistance phenotypes and genotypes has been documented in both clinical and environmental isolates, suggesting that hospital and environmental water sources can serve as silent reservoirs for antimicrobial resistance genes.

(Deekshit, & Srikumar, 2022; Fernandes et al., 2016) Although these ARGs may be phenotypically silent, they can be reactivated during host infection, potentially leading to challenges in treatment with current antibiotics or treatment failure in the future.

The prevalence of Gram-negative bacteria, detected ARGs, and observed trend in antibiotic resistance in this study raise concerns regarding the safety of water sources and bacterial contamination on hospital material surfaces, Therefore, continuous surveillance is necessary. The findings presented herein provide valuable information to support sustainable water management and the development of enhanced strategies to mitigate the potential rise of antibiotic-resistant bacteria within the community.

The limitation of this study is the relatively small bacterial sample size, which precluded the performance of statistical analyses. Larger sample sizes could provide more comprehensive information on bacterial diversity. In addition, the relative abundance of ARB and ARGs found in relation to total bacterial isolates could further elucidate the role of hospital material surfaces and environmental water as reservoirs and dissemination points for both ARB and ARGs. Future studies should also consider including hospital wastewater and soil samples to gain a more comprehensive understanding of the occurrence and distribution of ARBs and ARGs.

5. Conclusion

We report on the incidence of GNB and ARGs from environment. *Enterobacter* and *Klebsiella* were the most prevalent found in both hospital and water environments. bla_{SHV} and bla_{TEM} were highly prevalent in various species from both hospital and water, including *Klebsiella* and *Enterobacter* species. The results indicate that these environments may be reservoirs of ARGs and resistant bacteria. The prevalence of the same bacterial species and *bla* genes in both sectors suggests the cross-transfer of ARGs and resistant bacteria between different environments, hospital and water. These findings underscore the urgent need for routine surveillance of both hospital and environmental water sources to monitor ARB and ARG dissemination.

6. Acknowledgments

This study was supported by Thammasat University Research Fund, Contract No. TUFT74/2564. Meeprawat S. was supported by the Scholarship for Talented Students to Study Graduate Program in the Faculty of Science and Technology, Contract No. TB 12/2563. We also extend our gratitude to Thammasat University Hospital and Thammasat University for their assistance and cooperation in collecting environmental samples. Acknowledgments are also given to the Department of Biotechnology, Faculty of Science and Technology, Thammasat University, Rangsit Center. Special thanks to Assoc. Prof. Dr. Sutthirat Sitthisak, Department of Microbiology, Faculty of Medical Science, Naresuan University, for the positive control strains of the $bla_{\text{OXA-24}}$ and $bla_{\text{OXA-58}}$ genes.

Table 6 Antibiotic susceptibility test results (percentages) among GNB isolated from environmental water and hospital material surfaces

	Hospital material surfaces			Environmental water		
Antibiotics	**S**	**I**	**R**	**S**	**I**	**R**
AMP	22.86 (8/35)	11.43 (4/35)	65.71 (23/35)	27.08 (13/48)	6.25 (3/48)	66.67 (32/48)
AMC	54.29 (19/35)	11.43 (4/35)	34.29 (12/35)	62.50 (30/48)	6.25 (3/48)	31.25 (15/48)
CAZ	100.00 (35/35)	0.00 (0/35)	0.00 (0/35)	91.67 (44/48)	4.17 (2/48)	4.17 (2/48)
CTX	91.43 (32/35)	5.71 (2/35)	2.86 (1/35)	93.75 (45/48)	0.00 (0/48)	6.25 (3/48)
CRO	94.29 (33/35)	5.71 (2/35)	0.00 (0/35)	91.67 (44/48)	2.08 (1/48)	6.25 (3/48)
CXM	37.14 (13/35)	62.86 (22/35)	0.00 (0/35)	47.92 (23/48)	43.75 (21/48)	8.33 (4/48)
IPM	82.86 (29/35)	14.29 (5/35)	2.86 (1/35)	81.25 (39/48)	16.67 (8/48)	2.08 (1/48)
MEM	100.00 (35/35)	0.00 (0/35)	0.00 (0/35)	100.00 (48/48)	0.00 (0/48)	0.00 (0/48)
DOR	100.00 (35/35)	0.00 (0/35)	0.00 (0/35)	100.00 (48/48)	0.00 (0/48)	0.00 (0/48)
ATM	94.29 (33/35)	5.71 (2/35)	0.00 (0/35)	95.83 (46/48)	0.00 (0/48)	4.17 (2/48)

AMP: ampicillin; AMC: amoxicillin clavulanic acid; CAZ: ceftazidime; CTX: cefotaxime; CRO: ceftriaxone; CXM: cefuroxime; IPM: imipenem; MEM: meropenem; DOR: doripenem; ATM: aztreonam; S: susceptible; I: intermediate; R: resistant

Table 7 Antibiotic resistance patterns (percentages) of GNB isolated from environmental water and hospital material surfaces

Antibiotic resistance patterns	Total	Hospital material surfaces	Environmental water
AMC	8.43 (7/83)	2.86 (1/35)	12.50 (6/48)
AMP	40.96 (34/83)	34.29 (12/35)	45.83 (22/48)
AMP+AMC	18.07 (15/83)	28.57 (10/35)	10.42 (5/48)
AMP+AMC+CAZ+CTX+CRO+CXM+ATM	2.41 (2/83)	0.00 (0/35)	4.17 (2/48)
AMP+AMC+CTX+CRO+CXM	1.20 (1/83)	0.00 (0/35)	2.08 (1/48)
AMP+AMC+IPM	2.41 (2/83)	2.86 (1/35)	2.08 (1/48)
AMP+CXM	1.20 (1/83)	0.00 (0/35)	2.08 (1/48)
CTX	1.20 (1/83)	2.86 (1/35)	0.00 (0/48)

Note: AMP: ampicillin; AMC: amoxicillin clavulanic acid; CAZ: ceftazidime; CTX: cefotaxime; CRO: ceftriaxone; CXM: cefuroxime; IPM: imipenem; MEM: meropenem; DOR: doripenem; ATM: aztreonam

Table 8 Antibiotic resistance genes (ARGs) found (percentages) in GNB isolated from environmental water and hospital material surfaces

ARGs	Percentages of GNB with ARGs-positive	
	Hospital material surfaces	Environmental water
bla$_{AmpC}$	8.57 (3/35)	8.33 (4/48)
bla$_{SHV}$	91.43 (32/35)	75.00 (36/48)
bla$_{TEM}$	60.00 (21/35)	89.58 (43/48)
bla$_{NDM}$	17.14 (6/35)	52.08 (25/48)
bla$_{CTX-M}$	11.43 (4/35)	8.33 (4/48)
bla$_{OXA-24}$	28.57 (10/35)	25.00 (12/48)
bla$_{OXA-48}$	5.71 (2/48)	8.33 (4/48)
bla$_{OXA-58}$	11.43 (4/35)	33.33 (16/48)

Table 9 ARGs patterns found (percentages) in this study

ARGs patterns	Total	Environmental water	Hospital material surfaces
bla$_{NDM}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{OXA-24}$+*bla*$_{OXA-48}$	1.20 (1/83)	0.00 (0/48)	2.86 (1/35)
bla$_{SHV}$	9.63 (8/83)	2.08 (1/48)	20.00 (7/35)
bla$_{SHV}$+*bla*$_{AmpC}$	2.41 (2/83)	2.08 (1/48)	2.86 (1/35)
bla$_{SHV}$+*bla*$_{CTX-M}$	1.20 (1/83)	0.00 (0/48)	2.86 (1/35)
bla$_{SHV}$+*bla*$_{NDM}$+*bla*$_{OXA-24}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{SHV}$+*bla*$_{OXA-24}$	4.82 (4/83)	0.00 (0/48)	11.43 (4/35)
bla$_{SHV}$+*bla*$_{OXA-48}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$	4.82 (4/83)	8.33 (4/48)	0.00 (0/35)
bla$_{TEM}$ +*bla*$_{CTX-M}$+ *bla*$_{OXA-58}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{NDM}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{NDM}$+*bla*$_{OXA-48}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{OXA-58}$+*bla*$_{NDM}$	2.41 (2/83)	4.17 (2/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{OXA-24}$+*bla*$_{AmpC}$	1.20 (1/83)	0.00 (0/48)	2.86 (1/35)
bla$_{TEM}$+*bla*$_{OXA-58}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{OXA-58}$+*bla*$_{OXA-24}$	2.41 (2/83)	4.17 (2/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$	10.84 (9/83)	6.25 (3/48)	17.14 (6/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{AmpC}$	2.41 (2/83)	0.00 (0/48)	5.71 (2/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{CTX-M}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{NDM}$	16.87 (14/83)	20.83 (10/48)	11.43 (4/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{NDM}$+*bla*$_{AmpC}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{NDM}$+*bla*$_{CTX-M}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{NDM}$+*bla*$_{OXA-24}$	2.41 (2/83)	4.17 (2/48)	0.00 (0/35)

Table 9 Cont.

ARGs patterns	Total	Environmental water	Hospital material surfaces
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-24}$	7.23 (6/83)	2.08 (1/48)	14.29 (5/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-24}$+*bla*$_{CTX-M}$	1.20 (1/83)	0.00 (0/48)	2.86 (1/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-24}$+*bla*$_{CTX-M}$+*bla*$_{OXA-48}$	1.20 (1/83)	0.00 (0/48)	2.86 (1/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-24}$+*bla*$_{OXA-48}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$	3.61 (3/83)	4.17 (2/48)	2.86 (1/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{CTX-M}$	2.41 (2/83)	0.00 (0/48)	5.71 (2/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{NDM}$	6.02 (5/83)	6.25 (3/48)	5.71 (2/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{NDM}$+*bla*$_{AmpC}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{NDM}$+*bla*$_{OXA-24}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{NDM}$+*bla*$_{OXA-24}$+*bla*$_{AmpC}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{OXA-24}$	2.41 (2/83)	4.17 (2/48)	0.00 (0/35)
bla$_{TEM}$+*bla*$_{SHV}$+*bla*$_{OXA-58}$+*bla*$_{OXA-24}$+*bla*$_{OXA-48}$	1.20 (1/83)	2.08 (1/48)	0.00 (0/35)

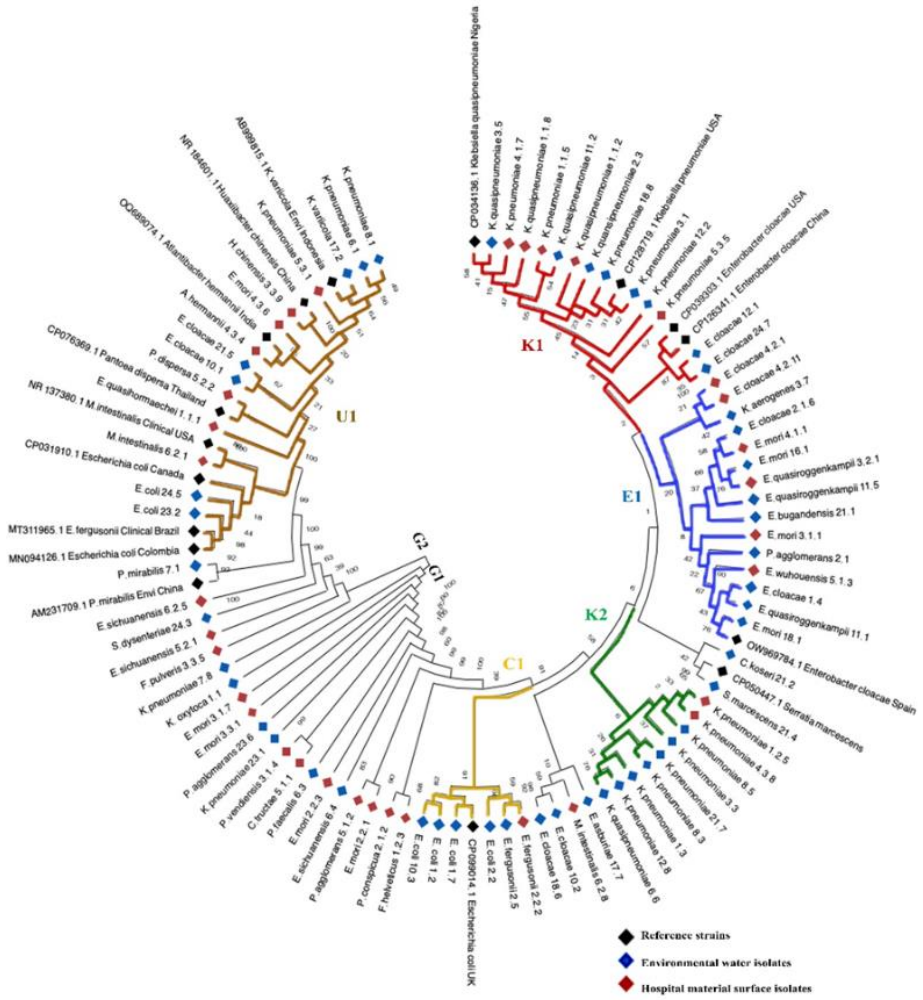

Figure 3 Phylogenetic tree inferred using the UPGMA method (Sneath, & Sokal, 1973), based on 83 bacterial 16*S* rRNA gene sequences and reference sequences. Bootstrap values (1000 replicates) are shown above branches. Evolutionary distances were calculated using the Maximum Composite Likelihood model (Tamura et al., 2004), with analysis performed in MEGA11 (Tamura et al., 2021)

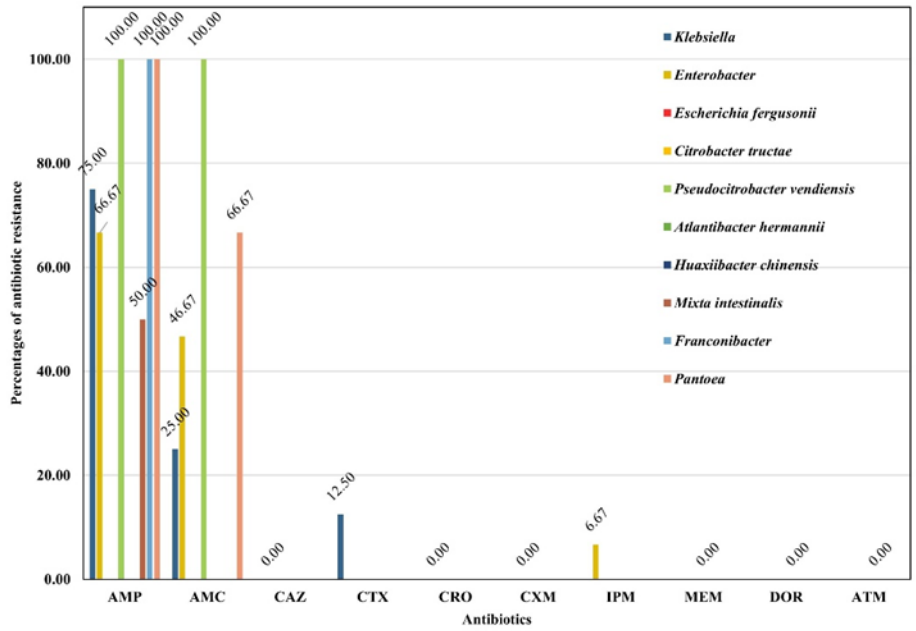

Figure 4 Antibiotic resistance profiles of Gram-negative bacteria isolated from hospital material surfaces

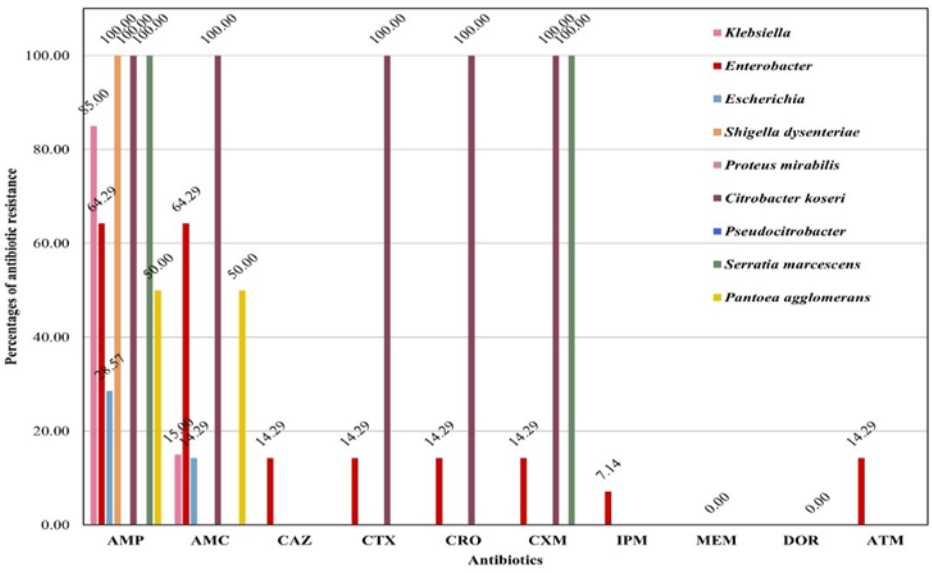

Figure 5 Antibiotic resistance profiles of Gram-negative bacteria isolated from environmental water sources

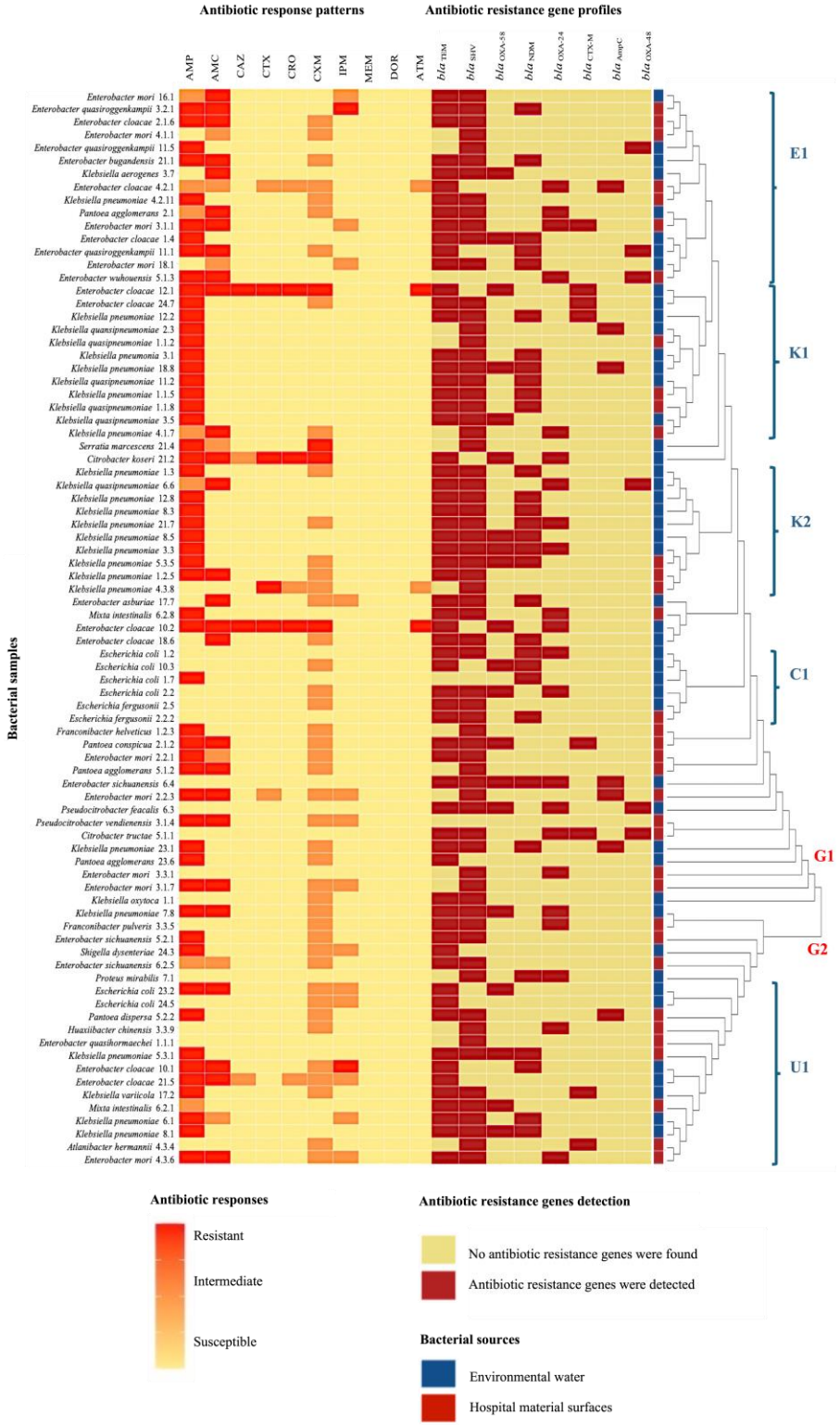

Figure 6 Heatmap illustrating antibiotic resistance patterns, ARG profiles, and phylogenetic relationships based on 16*S* rRNA gene sequences of all bacterial isolates

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