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ESBL-producing *Acinetobacter baumannii* from households near tertiary health care settings in Kerala: A one health surveillance study

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Abstract

Acinetobacter baumannii is a major multidrug-resistant nosocomial pathogen. The study investigated the prevalence and antibiotic resistance of *A. baumannii* in environmental (soil and water) samples from households near tertiary veterinary and human hospitals in Thiruvananthapuram, Thrissur, and Kalpetta, district of Kerala. Out of the 180 samples collected, 50 samples (27.8%) were confirmed to be positive for *A. baumannii* isolates, with significantly higher occurrence in households within one Km of the selected hospitals (up to 65% near Thrissur's human hospital), indicating distance-dependent dissemination likely from hospital effluents. The rate of occurrence of ESBL producers among the isolates phenotypically and genotypically was found to be 76 and 90%, respectively. The predominant ESBL-encoding gene harboured by the isolates was *bla_{SHV}* (82%). These findings highlight *A. baumannii*'s environmental persistence and role as an AMR reservoir near healthcare facilities, urging better wastewater management and surveillance to curb community spread of multidrug-resistant strains.

Keywords: *Acinetobacter baumannii*, ESBL producers, environmental samples, Kerala, hospital, households

1. Introduction

Antibiotics revolutionized medicine, enabling progress in surgery, organ transplants, cancer treatment, infection treatment and more. The need to survive causes microorganism to become resistant to drugs, which poses a major challenge to modern medicine (Nese *et al.*, 2021) [1]. Although antimicrobial resistance happens naturally, various factors have accelerated its rise. These include improper antibiotic use like self-medication or ceasing medication before completion, inappropriate or frequent antibiotic prescriptions by healthcare providers, widespread use in agriculture and animal husbandry, and the slow pace of developing new antibiotics (Ruiz-Pérez *et al.*, 2024) [2].

Pathogens resistant to antibiotics have spread globally, posing a significant threat to public health as they cause severe and life-threatening infections with rising rates of morbidity and mortality (Ting *et al.*, 2023) [3]. A significant number of antibiotic-resistant infections encountered in clinical settings are caused by a specific group of pathogens known as the 'ESKAPE' organisms, which include *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species. These pathogens are able to "escape" the effects of antibiotics and resist elimination through standard treatments. The main difficulty in managing infections caused by ESKAPE organisms lies in the prompt identification of their antibiotic resistance. A report from 2019 indicated that antimicrobial resistance was responsible for approximately 3.57 million deaths worldwide and among these, an estimated 0.5 to 1 million deaths were caused by carbapenem-resistant *A. baumannii* (Murray *et al.*, 2022) [4].

Among ESKAPE pathogens, *A. baumannii* is one of the main organisms causing disease in humans and animals. *A. baumannii* is an opportunistic Gram-negatives non-flagellated coccobacillus bacterium causing severe nosocomial infections.

This opportunistic pathogen is responsible for hospital and community-acquired infections. Its ability to form biofilms and resist desiccation, disinfectants, oxidative stress, and complement mediated killing are characteristics that allow *A. baumannii* to thrive within healthcare settings (Clémence *et al.*, 2022) ^[5].

The organism is mainly found as a hospital-associated pathogen, but it is increasingly found in environmental sources like wastewater, sewage, rivers, soil, animal manure, and sometimes on food, especially in areas influenced by human activity.

It survives well in moist environments and on dry surfaces, forming biofilms and persisting for long periods, which raises concerns about the environment acting as a reservoir for MDR strains that can spread to humans and animals. (Pulami *et al.*,2023) ^[6]

Acinetobacter baumannii's ability to resist antibiotics, survive in harsh environments, and lack of known toxin genes indicates that its virulence relies on a "persist and resist" approach. Its resistance to critical last-line antibiotics like colistin, tigecycline, and carbapenems has made it one of the most challenging hospital-acquired ESKAPE pathogens (Clémence *et al.*, 2022) ^[5]. There has been a global increase in the isolation of multidrug-resistant (MDR) and even pan-drug-resistant (PDR) strains of *A. baumannii*. Key resistance genes associated with β -lactamase production include *bla*_{TEM}, *bla*_{SHV}, *bla*_{GES}, *bla*_{PER}, *bla*_{VEB}, *bla*_{CTX-M}, *bla*_{KPC}, *bla*_{ADC}, and *bla*_{OXA}. Among beta-lactamases, carbapenem resistance is shown more prominently by the *A. baumannii*. (Kyriakidis *et al.*, 2021) ^[7].

The use of antibiotics in hospital settings significantly contributes to the growing challenge of antimicrobial resistance (AR). Studying the antibiotic resistance patterns of environmental isolates is important, as it helps uncover potential pathways through which antibiotics may be transferred from hospitals to the environment (Kunhikannan *et al.*, 2023) ^[8]. Increasing evidence indicated that human waste-related environments, including various municipal wastewater types such as domestic, industrial, hospital, and stormwater serve as reservoirs of resistant bacteria. Among these, hospital wastewater is considered a key source of clinically relevant *A. baumannii* (Hubeny *et al.*, 2021) ^[9]. The presence of *A. baumannii* in non-clinical environments like

water and soil further suggests that these habitats may act as reservoirs for antibiotic resistance genes (ARGs), particularly those encoding β -lactamases (Sykes *et al.*, 2024) ^[10].

2. Materials and Methods

2.1 Isolation and molecular confirmation of *A. baumannii*

A total of 180 environmental samples comprising soil (collected from 5-10 cm below the top layer) and water (well or tap water) from houses near the tertiary care veterinary and human hospitals from Thiruvananthapuram, Thrissur and Kalpetta districts of Kerala were collected. From each city, 30 households were selected, ten houses within one Km and five beyond one Km of veterinary and human hospitals. The study was conducted for a period of 10 months (November 2024 to August 2025). All the samples were aseptically collected and transported to the lab under chilled conditions. The samples were subjected to isolation and identification of *A. baumannii*. The soil samples were enriched at a rate of 1:10 dilution in buffered peptone water and incubated at 37 °C overnight. Enriched soil samples and water samples were streaked onto Leeds *Acinetobacter* agar. Colonies exhibiting characteristic pink mucoid colonies with pink colour diffused into the medium were selected (Fig 1.), and species-level identification was carried out using polymerase chain reaction (PCR) targeting the *gyrB* gene (Higgins *et al.*, 2007) ^[11] (Table 1. and Fig 2.). The DNA was extracted from all the isolates using the phenol-chloroform method and a PCR with the following conditions was carried out: 94 °C for 2 min, followed by 25 cycles of 94 °C for 1 min, 60C for 30 s and 72C for 1 min, with a final extension at 72C for 10 min. The amplicons were visualised following electrophoresis on a 1.5% agarose gel stained with ethidium bromide.

2.2 Detection of ESBL resistance

The Kirby-Bauer disc diffusion test, employing cefotaxime (CTX 30 µg), ceftazidime (CAZ 30 µg), ceftazidime/ clavulanic acid (CAC 30/10 µg), and cefotaxime/ clavulanic acid (CEC 30/10 µg) antibiotic discs, was used for the phenotypic detection of ESBL as per CLSI (2024) ^[12] standards. The PCR assay was employed to detect *bla*_{CTX-M}, *bla*_{TEM} and *bla*_{SHV} genes conferring ESBL resistance (Table 1.), following a method outlined in prior research (Bhoomika *et al.*, 2016) ^[13].

Table 1: Details of oligonucleotides used in the study

<i>gyrB</i>	F: 5'-GTTCTGATCCGAAATTCTCG-3' R: 5'-AACGGAGCTTGTCAAGGGTTA -3'	490	Higgins <i>et al.</i> , 2007
<i>bla</i> _{CTX-M}	F: 5'- CGCTTTGCGATGTGCAG-3' R: 5'-ACCGCGATATCGTTGGT-3'	550	
<i>bla</i> _{SHV}	F: 5'- GATGAACGCTTTCCCCATGATG-3' R: 5'-CGCTGTTATCGCTCATGGTAA-3'	214	Bhoomika <i>et al.</i> , 2016
<i>bla</i> _{TEM}	F: 5'- ATGAGTATTCAACATTTCCTG-3' R: 5'-GTCACAGTTACCAATGCTTA-3'	847	



Fig 1: Selective agar identification

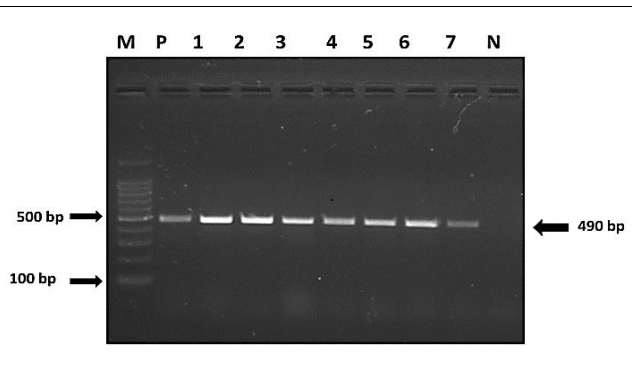


Fig 2: Molecular identification of *A. baumannii*.

3. Results and Discussion

Acinetobacter baumannii exhibits remarkable environmental persistence, enabling it to survive for extended periods often weeks to months on dry surfaces like hospital equipment, bedding, and healthcare worker attire, which facilitates nosocomial transmission. This resilience stems from its ability to tolerate desiccation, form protective biofilms, and resist common disinfectants such as chlorhexidine and phenols, outperforming many other Gram-negative bacteria in harsh conditions. (Gallego, 2016) [14].

Most of the studies in Kerala regarding the organism were done in clinical samples from human hospitals (Sathyanesan *et al.*, 2024 [15], Rudrapathy *et al.*, 2022 [16], (Rajan *et al.*, 2023) [17], Sheeba *et al.*, 2025) [18] etc. Studies conducted in animal samples are rare, with few studies, like that conducted by Hebball *et al.* (2025) [19], reporting the presence of *A. baumannii*. In the current study, out of the 180 samples collected, 50 samples (27.8%) were confirmed to be harbouring *A. baumannii*. Whereas study by Madalgi *et al.* (2025) [20] reported a lower rate of occurrence of the pathogen (5.5%) from water samples from Karnataka. These variations in prevalence may reflect differences in sampling methods, geographic locations, or environmental conditions influencing contamination levels.

Household environmental samples within one Km of selected

hospitals had a higher prevalence of *A. baumannii* isolates than those households that were farther away. Among the households within one Km, the ones near the human hospital in Thrissur showed the highest rate (65.0%), while the lowest (20.0%) were observed near the veterinary hospital in Thiruvananthapuram (Table 2.). Kalasseril *et al.* (2020) [21] found *A. baumannii* in 5.3% of the isolates from water bodies near hospitals across three districts of Kerala. These variations may be due to the difference in hospital type, patient load, and surrounding ecology which influenced difference in contamination patterns. Households beyond one km from the selected hospitals showed either no *A. baumannii* isolates or occurrence rates of only 10-20%. This indicated that environmental dispersion of the organism diminishes with increasing distance from the hospital. Statistically there was a significant difference in the distance wise occurrence of *A. baumannii* isolates from households near the two selected hospitals in Thrissur. Zone-wise, the highest *A. baumannii* occurrence rate came from households near Thrissur's human hospital (50.0%), while the lowest was near Thiruvananthapuram's veterinary hospital (16.67%) (Table 3.). These differences likely stem from factors like urban overcrowding, varying disease prevalence, and inconsistent sanitation practices across the cities.

Table 2: Comparison of occurrence *A. baumannii* within and beyond 1Km

Place	Vet hospital/ human hospital	Within 1Km (n = 20)	Beyond one Km (n = 10)	P-value
Kalpetta	Vet hospital	6 (30.0)	0	0.074 ^{ns}
	Human hospital	6 (30.0)	0	0.074 ^{ns}
TVM	Vet hospital	4 (20.0)	1 (10.0)	0.640 ^{ns}
	Human hospital	6 (30.0)	0	0.074 ^{ns}
Thrissur	Vet hospital	11 (55.0)	1 (10.0)	0.024*
	Human hospital	13 (65.0)	2 (20.0)	0.050*

* Significant at 0.05 level; ns non-significant

Table 3: Comparison of occurrence *A. baumannii* nearby veterinary hospital and human hospital between different locations

Place	Vet hospital		Human Hospital	
	Total sample	No (%) of PCR positive	Total sample	No (%) of PCR positive
Kalpetta	30	6 (20.00)	30	6 (20.00 ^b)
TVM	30	5 (16.67)	30	6 (20.00 ^b)
Thrissur	30	12 (40.00)	30	15 (50.00 ^a)
Chi square value (p-value)	5.023 ^{ns} (0.081)		8.571* (0.014)	

* Significant at 0.05 level; ns non-significant; Percentage having different letter as superscript differ significantly

3.1 ESBL-producing *A. baumannii*

In the study, out of the 50 *A. baumannii* isolates, 76.0% of the isolates were classified as ESBL producers phenotypically because they demonstrated synergistic effects between third-generation cephalosporins and their respective clavulanate combinations. The resistance shown by the isolates towards ceftazidime and cefotaxime antibiotics was 78.0 and 82.0%, respectively. Highest rate of occurrence of ESBL-producing *A. baumannii* isolates was observed in the households near human hospital in Thiruvananthapuram (Table 4. And Fig 3.). Yusuf *et al.* (2023) [22] reported a lower rate of occurrence (25%) of ESBL-producing *A. baumannii* isolates recovered from the waste water from residential areas in Nigeria. A study conducted by Hossain and Dey (2023) [23] reported the resistance rate of *A. baumannii* isolates recovered from the soil samples from the hospitals in Dhaka towards ceftazidime was 75.0%. The higher prevalence of ESBL-producing *A. baumannii* near hospitals points to contamination spread via proximity, with effluents and vectors carrying strains to nearby homes. This highlights the pressing need for stronger control strategies and monitoring efforts.

Genotypically, 90% of the *A. baumannii* isolates were identified as ESBL producers as they harboured any one of the three ESBL-encoding genes viz., *bla*_{CTX-M}, *bla*_{SHV}, and *bla*_{TEM} considered in the study. Most of the isolates harboured *bla*_{SHV} gene (82.0%). Genotypically highest rate of detection of ESBL-producing *A. baumannii* was seen in households near human hospital in Thiruvananthapuram and veterinary hospital in Thrissur (Table 5. And Fig 4.). Rahman (2024) [24] conducted a study in waste water samples in Dhaka city and the most prevalent ESBL-encoding gene in the *A. baumannii* isolates was *bla*_{TEM} (55.6%). A study conducted in lagoon waters from Nigeria by Ajoseh *et al.* (2025) [25] reported that 25.0% of the isolates were characterised as ESBL producers. These genes play a critical role in bacterial drug resistance by producing enzymes that hydrolyze beta-lactam antibiotics. Mutations in these genes expand the enzyme's substrate range. Often plasmid-mediated, ESBL genes spread rapidly via horizontal transfer and co-carry resistance to other drugs like aminoglycosides, complicating therapy and increasing nosocomial infection risks. This indicates there is an urgent need to combat this issue.

Table 4: Distance-wise phenotypic characterisation of *A. baumannii* isolates from houses near veterinary and human hospital from three cities in Kerala for ESBL production

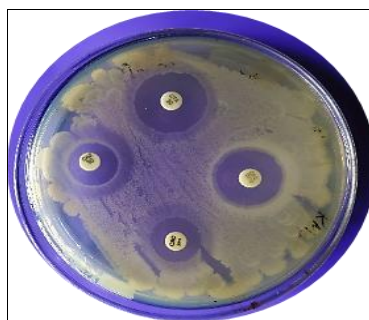
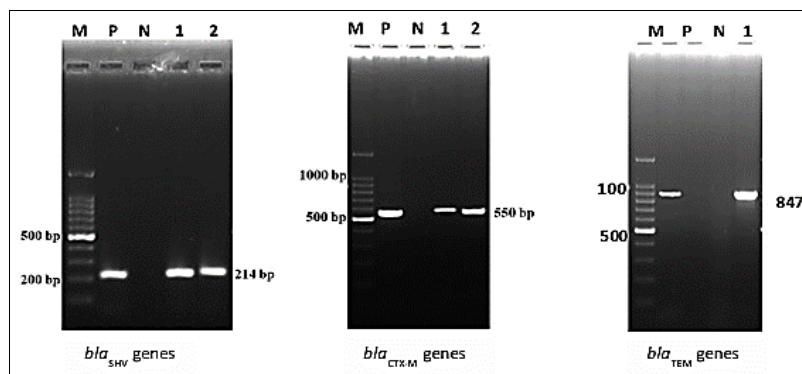
SL No.	Location	Distance	No. of <i>A. baumannii</i> isolates	ESBL producers		CAZ		CTX	
				No	p value	S	R	S	R
Veterinary									
1	Kalpetta	Within 1Km	6	5(83.3)	-	1(16.7)	5(83.3)	2(33.3)	4(66.7)
		Beyond 1Km	0	0		0	0	0	
2	TVM	Within 1Km	4	2(50)	-	1(25)	3(75)	0	4(100)
		Beyond 1Km	1	0		1(100)	0	1(100)	0
3	Thrissur	Within 1Km	11	10(90.9)	0.167	0	11(100)	0	11(100)
		Beyond 1Km	1	0		1(100)	0	1(100)	0
Human									
4	Kalpetta	Within 1Km	6	5(83.3)	-	1(16.7)	5(83.3)	2(33.3)	4(66.7)
		Beyond 1Km	0	0		0	0	0	0
5	TVM	Within 1Km	6	6(100)	-	0	6(100)	0	6(100)
		Beyond 1Km	0	0		0	0	0	0
6	Thrissur	Within 1Km	13	10(76.9)	0.095	4(30.8)	9(69.2)	1(7.7)	12(92.3)
		Beyond 1Km	2	0		2(100)	0	2(100)	0
		Total	50	38(76)		11(22)	39(78)	9(18)	41(82)

Values in the brackets are percentages out of total PCR positive cases in each type ns= non-significant, S- Sensitive, R- Resistant, CAZ- Cefotaxime, CTX-Cefotaxime

Table 5: Distance-wise genotypic characterisation of *A. baumannii* isolates from houses near veterinary and human hospital from three cities in Kerala for ESBL production

Sr No.	Location	Distance	No. of <i>A. baumannii</i> isolates	Isolates harboring ESBL genes					
				<i>bla</i> _{SHV}		<i>bla</i> _{CTX-M}		<i>bla</i> _{TEM}	
				No.	p-value	No.	p-value	No.	p-value
Veterinary									
1	Kalpetta	Within 1Km	6	4(66.7)	-	3(50)	-	3(50)	-
		Beyond 1Km	0	0		0			
2	TVM	Within 1Km	4	4(100)	-	2(50)	-	1(25)	-
		Beyond 1Km	1	0		0			
3	Thrissur	Within 1Km	11	10(90.9)	0.917	9(81.8)	0.833	9(81.8)	0.833 ^{ns}
		Beyond 1Km	1	1(100)		1(100)			
Human									
4	Kalpetta	Within 1Km	6	4(66.7)	-	4(66.7)	-	2(33.3)	-
		Beyond 1Km	0	0		0			
5	TVM	Within 1Km	6	5(83.3)	-	4(66.7)	-	6(100)	-
		Beyond 1Km	0	0		0			
6	Thrissur	Within 1Km	13	11(84.6)	0.524	7(53.8)	0.733	9(69.2)	0.629 ^{ns}
		Beyond 1Km	2	2(100)		1(50)		2(100)	
Total			50	41(82)		31(62)		33(66)	

* Significant at 0.05 level; ns=non-significant, Values in the brackets are percentages out of total PCR positive cases in each type

**Fig 3:** Phenotypic detection of ESBL producers**Fig 4:** Molecular identification of ESBL encoding genes (Lane M: Marker, Lane N: Negative control, Lane P: Positive control, Lane 1-2: Sample)

4. Conclusion

The current study revealed the significant environmental dissemination of *A. baumannii* from tertiary veterinary and human hospitals in Kerala, where isolation rates were markedly higher from households within one Km of the selected hospitals demonstrating distance-dependent contamination gradients driven by hospital effluents, vectors, and poor sanitation. The ESBL-producing *A. baumannii* is important because it can inactivate commonly used beta-lactam antibiotics, survive for long periods in soil and water, and spread resistance genes to other bacteria, making infections difficult to treat. Through contaminated environmental sources such as wastewater, soil, and surface water, it can infect or colonise in humans and animals, facilitating a continuous transmission cycle between hospitals, communities, livestock, and the environment. The ESBL-producing *A. baumannii* were isolated more from the households in close proximity with the selected hospitals. These results highlighted the fact that households near hospitals can act as sentinel sites for monitoring AMR reservoirs, emphasizing the "persist and resist" strategy of *A. baumannii*. The findings of the current study call for urgent interventions including enhanced wastewater treatment, antibiotic stewardship, and routine environmental surveillance to curb transmission from clinical to community settings, mitigating the escalating threat of multi/pan-drug resistant strains in Kerala.

5. Conflict of interest

The authors declare that there are no actual or potential conflicts of interest that could have influenced this work inappropriately.

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