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Extended Spectrum Beta-Lactamase Production by *Acinetobacter baumannii* Isolated from Hospital Environment

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Abstract

Extended spectrum β-lactamases production was determined by Double Disc synergy test for twenty-one bacterial isolates of Acinetobacter bumannii isolated from the hospital environment. This study investigates the prevalence, genetic mechanisms, and clinical implications of ESBL production by A. baumannii. We employed molecular techniques to identify and characterize ESBL genes in clinical isolates, alongside evaluating their susceptibility profiles against a range of antibiotics. These resistant strains were notably associated with severe infections and poor clinical outcomes. The results underscore the urgent need for robust surveillance and novel therapeutic strategies to address the growing threat of ESBL-producing A. baumannii. The results showed 38.09% (8/21) of isolates were ESBL-positive, distributed between 33.33% (4/12) ESBL-positive environmental A. baumannii isolates and 44.44% (4/9) ESBL-positive clinical A. baumannii isolates. Based on statistical analysis, there was no significant correlation found between the isolates source and the production of ESBL (P > 0.05). Detection of gene encoding a class A extended spectrum β-lactamase blaPER-1, Integron genes (int1, and int2 genes) in 19 different environmental and clinical A. baumannii isolates by polymerase chain reaction technique revealed genes frequencies were 42.1% (8/19), 100% (19/19), and 57.89% (11/19), respectively. The results analysis exhibited that the frequency of genes had no significant differences (P > 0.05) between environmental and clinical isolates of A. baumannii.

Keywords: *Acinetobacter baumannii*, extended spectrum beta-lactamase, *bla*per-1 gene, antibiotic susceptibility, genetic mechanisms

INTRODUCTION

Acinetobacter baumannii is an increasingly significant common ESBL generating bacteria, making their eradication challenging. A class of group A β-lactamases is known as ESBLs, play an important role in resistance against later generation cephalosporins, such as cefotaxime, ceftazidime, and cefepime, while cephamycin and carbapenems are exempt from their effects. One distinguishing feature of ESBLs is their ability to be suppressed by β-lactamase inhibitors, such as clavulanic acid; this property is utilized in ESBL detection assays (Litake et al., 2015; Safari et al., 2015) [1, 2]. Even though phenotypic responses can vary, it is advised to look for ESBLs even if they just cause low level resistance. In routine clinical diagnostics, the need for quick and accurate techniques still exists. No β-lactam medications are currently being developed that can avoid the β-lactamases and effectively treat the deadly *A. baumannii* infection. Additionally, many ESBL-

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producing isolates have co-resistance to other antibiotic families which supports the necessity for routine ESBL identification in diagnostic processes (Litake et al., 2015) [1].

The majority of the ESBL genes found in Acinetobacter species are of the VEB or PER types, however reports have also included derivatives of TEM, SHV, GES, and CTX-M. (Abd El-Baky et al., 2020) [3]. ESBLs are easily transmitted from one organism to another because they are found on integrons and are mostly mediated by plasmids (Abdar et al., 2019) [4]. The blaPER-1 gene encoding a class A extended spectrum β-lactamase *bla*PER-1 is widespread in the strains of Acinetobacter *spp*. In most cases, the study of the *bla*PER-1 gene in different Gram-negative bacteria revealed both the chromosomal and plasmid locations (Sacha et al., 2012) [5]. In *A. baumannii*, integrons, transposons, and plasmids all significantly aid in the acquisition and transfer of an antibiotic-resistant trait. According to Monfared et al. (2019) [6], integrons are important because they can express and carry resistance genes. The nosocomial spread of isolates and multidrug resistances have been closely linked to class I and class II integrons, as has been shown in both environmental and clinical strains of *A. baumannii* worldwide (Almasaudi, 2018) [7]. Integron genes are essential in the horizontal transmission of genetic elements and antibiotic resistance. Either the host plasmid or the bacterial chromosome includes resistance genes. The integrase protein is encoded by the *intI* gene and is a member of the tyrosine-recombinase family. Recombination of distinct DNA molecules into gene cassettes is a function of the enzyme integrase. Super integrons and mobile integrons, which disseminate the genes that cause drug resistance, are the two subsets of integrons. Integrons have the capacity to ensnare the genes for the antibiotic resistance cassettes that cause the spread of MDR and reduce the number of infection-treating choices (Al-Kadmy et al., 2020) [8].

MATERIAL AND METHODS

Baumannii Isolates

Twenty-one (21) *A. baumannii* were recovered from environmental and clinical swabs which included 12 of *A. baumannii* isolates recovered from environmental swabs and 6% (9/150) of *A. baumannii* isolates recovered from clinical swabs.

Phenotypic Detection of Esbl by Double Disc Synergy Test

Young colonies of *A. baumannii* isolates were cultured on brain heart infusion agar, and the turbidity was adjusted to match the 0.5 McFarland turbidity standard. After being dipped into the modified suspension, a sterile cotton swab was streaked across the Mueller-Hinton agar plate surface. To ensure an even distribution of inoculum, the streaking was repeated twice more while the plate was rotated by about 60° each time. A disk of ticarcillin-clavulanate was then placed in the center of the Muller hinton agar medium, 20 mm away from the cefepime, cefotaxime, and ceftazidime, and the plates were left for another 15 to 20 minutes at room temperature to dry. Phenotypic proof of ESBL generation is considered to have occurred when there is a discrepancy of at least 5 mm between the zone diameters of the cephalosporin disks and the corresponding cephalosporin/clavulanate disks (Ghaima, 2018) [8].

Detection of *Bla*per-1, *Int1* Gene, and *Int2* Genes

Using the GspinTM Genomic DNA Extractions Kit, genomic DNA was isolated from bacterial isolates of *A. baumannii* in accordance with the manufacturing company's (iNtRON/Korea) instructions. The Nanodrop RNA/DNA spectrophotometer was used to measure the concentration and purity of isolated DNA. Primers from Scientific Researcher **Corporation** Ltd. in Iraq were used in this study to identify the target genes in *A. baumannii* isolates. *blaPER*-1 (F5'-GCAACTGCTGCAATACTCGG-3', R5'-ATGTGCGACCACAGTACCAG-3'), *int1* (F5'-GGTGTGGCGGGCTTCGTG-3', R5'-GCATCCTCGGTTTTCTGG-3'), and *int2* (F5'-CAC GGATATGCGACAAAAAGGT-3', R5'-GTAGCAAACGAGTGACGAAATG-3') (Peymani et al., 2012; Monfared et al., 2019) [10, 6]. PCR cycling conditions protocol for these genes were installed in Table 1. Using agarose gel electrophoresis, the PCR results were examined. 1.5% Agarose gel was

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made by dissolving it in a water bath at 100°C for 15 minutes using 1X TBE, then letting it cool to 50°C. To the agarose gel solution, 3 µL of ethidium bromide dye was added. Once the comb was properly positioned, agarose gel solution was added to the tray. The comb was then carefully removed from the tray and allowed to solidify for fifteen minutes at room temperature. After being fixed in the electrophoresis chamber, the gel tray was filled with 1X TBE buffer. In each comb well, 10 µl of the PCR product was added, and in the first well, 3 µl of the 100 bp ladder. For one-hour, electric current was run at 80 AM and 100 volts. UV transilluminator was utilized to visualize the results of PCR.

Table 1. PCR thermo-cycler conditions protocol.

Gene Name	Initial denaturation Temp./time	Denaturation Temp./time	Annealing Temp./time	Extension Temp./time	Cycle	Final extension Temp./time
*bla*PER-1	95°C/4 min.	95°C/30 sec.	59°C/30 sec.	72°C/40 sec.	32	72°C/5 min.

STATISTICAL ANALYSIS

IBM Statistical Package for Social Sciences (SPSS, United States) Statistics software, version 27, was used to conduct a Chi square test (χ^2) for statistical analysis to evaluate the independence of the variables. Statistical significance was defined as $P < 0.05$ (Daniel, 2009) [11].

RESULTS AND DISCUSSION

ESBL production by *A. baumannii*

Phenotypic testing in current investigation (Figure 1) showed 38.09% (8/21) of *A. baumannii* isolates were ESBL-positive, distributed between 33.33% (4/12) ESBL-positive environmental *A. baumannii* isolates and 44.44% (4/9) ESBL-positive clinical *A. baumannii* isolates. A significant correlation between the isolates' source and the production of ESBL was not found according to the findings analysis ($P > 0.05$) as shown in (Table 2). One of the most significant resistance mechanisms used by *A. baumannii* strains is the production of ESBLs. Many ESBL makers may go undetected because of the challenges in detection. One of the most widely used and reliable methods for confirming the presence of ESBL is the Double Disc Synergy test, which relies on the synergistic interactions between cephalosporin and clavulanate when clavulanic acid is present. The increase in zone diameter is due to clavulanic acid's suppression of β-lactamase (Khan et al., 2019) [12]. Cefepime, one of the studied cephalosporins, has the highest sensitivity for ESBL detection. Cefotaxime was the other cephalosporin in current investigation that was successful in identifying ESBL. Ceftazidime, on the other hand, was discovered to have a low sensitivity of identifying ESBLs.

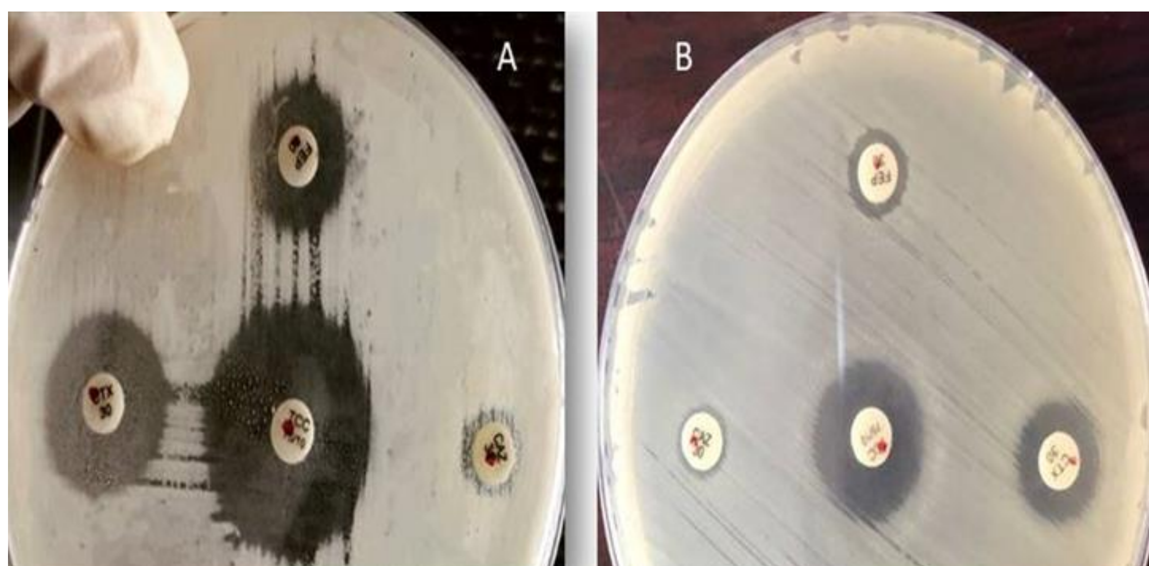

Figure 1. Phenotypic detection of ESBL in *A. baumanii* isolates by Double Disc synergy test. A: positive result, B: negative result.

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Table 2. Percentage of ESBL production by *A. baumannii.*

Source of Isolate	**Total No.**	**Positive Isolates**	
		No.	**%**
Environmental	12	4	33.33
Clinical	9	4	44.44
Total	21	8	38.09
χ^2		0.016	
P value		0.899*	

Local study by Ghaima (2018) [8] showed that 61.53% of clinical *A. baumannii* was isolated from burns were ESBL producer. A study of (Ranjbar & Farahani, 2019) [13] showed 85% of clinical *A. baumannii* isolated from burns were ESBL-positive. A study of (Namiganda et al., 2019) [14] showed only three strains (15.79%), out of 19 tested clinical *A. baumannii* isolates were ESBL-positive. A study by Kaur & Singh (2018) [15] reported ESBL production in *A. baumannii* was 27.5%. A study of Alkasaby & El Sayed Zaki (2017) [12] demonstrated ESBLs production in 2.1% clinical *A. baumannii* isolates. A study of Fallahe et al. (2014) [16] identified 84.2% of *A. baumannii* isolates as ESBL producers by phenotypic method. The results of various studies can differ depending on the areas in which they were conducted, the prevalence of pathogenic strains carrying resistance genes, particularly in hospitals, patient abuse or overuse of antimicrobial drugs, sample size, different antibiotic usage in different screening method (Kaur & Singh, 2018; Abdar et al., 2019) [4, 15]. The widespread use of third-generation cephalosporins like cefotaxime, ceftriaxone, and ceftazidime, had been led to emergence of novel beta lactamases like the ESBLs. Antibiotics' selection pressure makes it easier for foreign resistance genes to adapt to bacteria. Because Acinetobacter *spp*. is continuously exposed to these antibiotics in hospital settings, ESBLs ought to have spread widely among these species (Kaur & Singh, 2018) [15]. ESBL genes are frequently found on class 1 integrons and are mostly mediated by plasmids, so they are easily transferred to other bacteria. ESBL-producing strains spread more quickly because of this genetic exchange among bacteria. However, the presence of inhibitor-resistant β-lactamase clavulanic acid obscures the inhibitory action and causes an ESBL detection test to return a false negative result (Litake et al., 2015) [1].

Detection of *bla*PER-1, *int1, and int2 genes*

PCR technique showed the frequency of *bla*PER-1, *int1*, and *int2* genes in 19 different environmental and clinical *A. baumannii* isolates were 42.1%, 100%, and 57.89% respectively. The results analysis exhibited that the frequency of genes had no significant differences (P > 0.05) between environmental and clinical *A. baumannii* as shown in the Table 3.

Table 3. Frequency of genes according to the source of isolates.

Source of Isolate	**No. (%)**		
	BlaPER-1	***int1***	***int2***
Environmental (12)	6(50)	12(100)	5(41.66)
Clinical (7)	2(28.57)	7(100)	6(85.71)
Positive no.	8(42.1)	19(100)	11(57.89)
χ^2	0.833	0	3.51
P value	0.361*	1*	0.06*

In the current investigation *bla*PER-1 gene was found in 8 bacterial isolates of *A. baumannii* with product size 340 base pair as shown in Figure 2. Different studies noted different prevalence for blaPER-1 in *A. baumannii*, 31.4% in a study of Khoshnood et al. (2020) [17], 38.3% in Yang et al. (2019) [18], 13.3% in Ghasemi et al. (2018) [19], 6.3% in Sung (2018) [20], 48% in Bardbari et al. (2017) [21], and 30.2% in study of Qi et al. (2016) [22]. Moreover, all four environmental and two clinical *A. baumannii* isolates that produced ESBLs in current study had *bla*PER-1gene which

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indicated that *bla*PER-1gene is the prevalent ESBL genotype among *A. baumannii* strains, whereas two negatives clinical ESBLs producers were had *bla*PER-1gene. *A. baumannii* isolates have been reported to contain both ESBLs and AmpC-type ß-lactamases; however, the coexistence of both enzyme types in a single strain increases the possibility of false-negative test results for the identification of ESBLs. Clavulanate and cephalosporins work synergistically to suppress ESBL, however AmpC-type ß-lactamases are resistant to this effect, which is the most likely explanation (Abdar et al., 2019) [4]. A study by Fallah et al. (2014) [16] found 78.03% of ESBLs *A. baumannii* isolates contained *bla*PER1 gene.

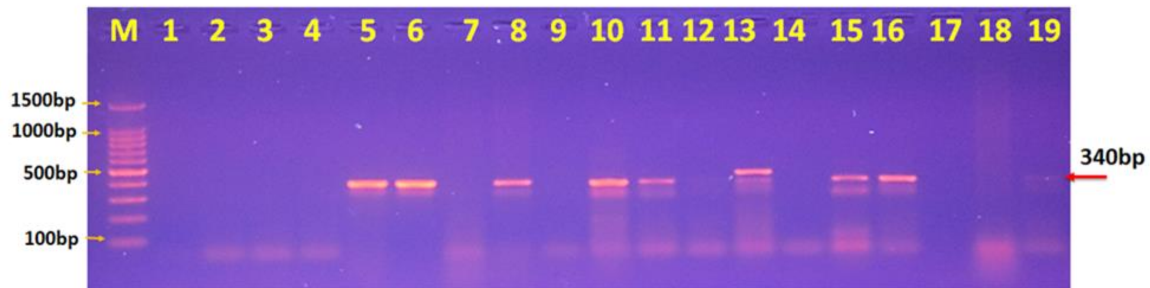

Figure 2. Agarose gel electrophoresis image that showed PCR product analysis of *blaPER-1* gene in *Acinetobacter baumannii* isolates. M (Marker ladder 1500-100 bp). Lane (5,6,8,10,11,13,15,16) showed some positive *blaPER-1* gene at 340 bp product size.

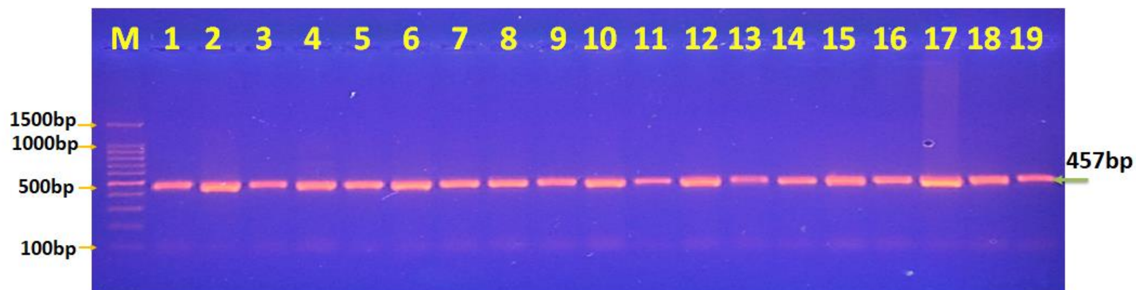

Figure 3. Agarose gel electrophoresis image that showed PCR product analysis of antibiotic resistance gene *int1* gene in *A baumannii* isolates. M (Marker ladder 1500-100 bp). Lane (1-19) showed positive *int1* gene at 457 bp product size.

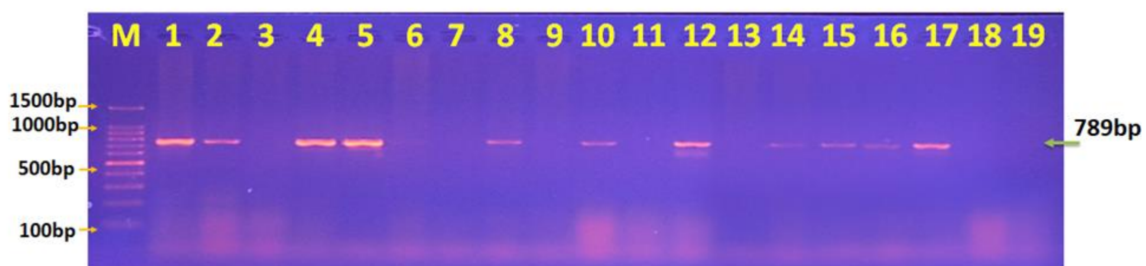

Figure 4. Agarose gel electrophoresis image that showed PCR product analysis of antibiotic resistance gene *int2* gene in *Acinetobacter baumannii* isolates. M (Marker ladder 1500-100 bp). Lane (1,2,4,5,8,10,12,14,15,16,17) showed some positive *int2* gene at 789 bp product size.

PCR results showed that integron class I gene was found in all isolates with product size 457 base pair as shown in Figure 3, while integron class II gene found in 11 bacterial isolates of *A. baumanii* isolates with product 789 base pair as shown in Figure 4. The high prevalence of integron class I gene in *A. baumannii* isolates in current study can be attributed to several factors, including the improper

use of antibiotics for *A. baumannii* infections; the ability of integrons to obtain new gene cassettes; and the spread of antibiotic resistance among clinical isolates (Mahmood, 2022) [23]. Additionally, Class 1 integrons are more common than integrons of other classes, most likely due to their presence on genetic elements like transposons and conjugative plasmids (Al-Shaabani et al., 2020) [24]. Another studies noted different percentages for *int I* and *int II* genes in *A. baumannii*, a study by (Al-Asady, 2021) [25] recorded 80% (16/20) class I integron and no isolate contain class II integrin, a study by Xu et al. (2020 [26] showed 13.51% (10/74) Class I integron and no Class II integron was detected in *A. baumannii*, a study by Halaji et al. (2018) [27], mentioned that Class I and II integrons were detected in 63.9% and 78.2% of the *A. baumannii* isolates, respectively. A study by Deylam Salehi et al. (2017) showed that 25.7% and 88.6% of the *A. baumannii* isolates carried *int I* and *int II* genes respectively, a study by Amin et al. (2019) [28] revealed that among 77 MDR *A. baumannii* isolates, 34 had *int I* and 10 had int II genes, a study by Zeighami et al. (2019) [29] recorded 67% Class I integron and 10% class II integron genes out of 100 *A. baumannii* isolates, a study by Ardeshiri et al. (2017) [30] recorded the class 1 and class 2 integrons were 63.5% and 53.8%, respectively in *A baumannii* isolates. A study by Deylam Salehi et al. (2017) [31] recorded that the prevalence of class I, II integrons genes was 25.7% and 88.6% respectively. It is noted these results are at odds with current study findings, which may be due to the different sample sources, levels of hygiene, and geographical locations (Deylam Salehi et al., 2017) [31].

CONCLUSION

ESBL producing *A. baumannii* isolates were detected in the hospital environment. *bla*PER1gene is the prevalent ESBL genotype among *A. baumannii* strains. Integron class I gene is widespread among *A. baumannii* isolates.

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