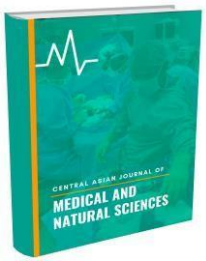

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Article

Identifying the Extended Spectrum β-Lactamase in Acinetobacter Baumannii From Wounds

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Abstract: Extended spectrum β-lactamases (ESBLs) are enzymes encoded by mobile genes that confer resistance to many antibiotics by hydrolyzing cephalosporins and penicillins. Infections caused by Gram-negative bacteria like Acinetobacter baumannii, which can produce ESBLs, pose significant treatment challenges and are linked to increased morbidity and mortality. However, data on ESBL-producing A. baumannii in clinical specimens remains limited. This study aimed to identify A. baumannii isolates from clinical samples and assess their antibiotic resistance, particularly ESBL production. One hundred clinical specimens collected between October 2023 and May 2024 were cultured and analyzed using biochemical assays. Sixteen A. baumannii isolates were identified, with only two producing ESBLs. These ESBL-producing isolates showed high resistance to multiple antibiotics, including amoxicillin (100%) and cefotaxime (87.5%). The findings highlight the critical need for vigilant infection control and antibiotic stewardship to prevent the spread of multidrug-resistant A. baumannii in clinical settings.

Keywords: ESBL, Acinetobacter baumannii, Antibiotic resistance, Multidrug-resistant bacteria, Infection control

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1. Introduction

The management of patients in hospitals and communities is currently faced with a significant therapeutic challenge. This is due to a complex and rapidly evolving group of enzymes called extended-spectrum β-lactamases (ESBLs), which are plasmid-mediated [1]. These enzymes are encoded by movable genes [2]. These often genes encode resistances to a variety of antibiotics, such as sulfamethoxazole-trimetroprime, amino glycosides, tetracyclines, fluoroquinolones, and cephalosporins [3]. Liakopoulos et al. (2016) state that ESBL can hydrolyze extended-spectrum cephalosporins that have an oxymino side chain. These cephalosporins include ceftriaxone, cefotaxime, cephpodoxime and ceftazidime. Acinetobacter baumannii is an opportunistics pathogen that has become more common over the past 20 years.

It is a significant species that plays a role in nosocomial infections, which can include wound infections, pneumonia, urinary tract infections, and septicemia [4,5]. The use of broad-spectrum antibiotics has increased during the past several years due to an increase in isolates that have been shown to be resistant to numerous medications. It is a major therapeutic challenge to treat infections caused on by this bacterium [6]. Reports have mostly identified Ambler class A clavulanic acid-inhibited ESBLs as the majority of

Acinetobacter expanded-spectrum β-lactamases [7]. Aim of this investigation was to determine ESβLs enzyme and assess antibiotic susceptibility in Acintobacter baumannii that was isolated from clinical wound specimens in Nasiriyah City.

2. Materials and Methods

Samples Collection

All Samples collected from patients hospitalized or readmitted to Nasiriyah Hospital in Nasiriyah City between October 2023 and May 2024 were used in this study. Samples (n = 100) were taken from wounds using swabs.

Identification of Acintobacter

The specimens in the microbiology lab were grown on MacConkey agar and incubated under aerobic conditions for a whole night at 37°C in bacteriological incubators ,based on the morphological characteristics of the colonies and a Gram stain microscopy analysis. The Acintobacter isolates were diagnosed using biochemical tests such as Oxidase, Urease, Methyl red, Voges proskauer, Indole, Simon citrate, Kliglers iron agar tests, and string test in order to distinguish Acintobacter from other gram negative bacteria.

Bacterial Susceptibility Detection

Each isolate underwent a susceptibility test against sixteen antibacterial agents (Bioanalyse, Turkey). Based on the Kirby-Bauer disc diffusion method, the testing findings determined the interdependence among the different species of Acintobacter baumannii (Kumari et al., 2023). The antibiotic disc inhibition zone was evaluated in accordance with CLSI recommendations (2014). Amikacin 30µg (AK), Azithromycin 30µg (ATM), Gentamicin 10µg (GN), Netilmicin 30µg (NET), Imipenem 10µg (IMP), Meropenem 10µg (MEM), Ceftazidim 30µg (CAZ), Ceftriaxone 30µg (CRO), Cefotaxime 30µg (CTX), Ciprofloxacin 5µg (CIP), Norfloxacin 10µg (NOR), Naldixic acid 30µg (NA), Carbencillin 100µg (PY), Amoxicillin-clavulanic acid 20µg (AUG), Amoxicillin 10µg (AX) and Nitrofurantion 300µg (F)

ESBLs detection

A sterile swab was dipped into the suspension of A. baumannii suspinsion, the turbidity met 0.5 McFarland standards, aftar that, Mueller-Hinton agar plate was inoculated in three different directions. An amoxicillin-clavulanate disc 30µg was put in the middle of the agar plate. One disc containing 30µg ceftazidime was set 25mm away of amoxicillin-clavulanate, while cefotaxime 30µg was put 25mm away on the other side of amoxicillin-clavulanate. After an overnight incubation period at 37°C, the plates were considered to have produced ESBLs if inhibition zone of cefotaxime, clavulanate, amoxicillin-clavulanate disc, or both, expanded [8].

3. Results

Our results showed there are 16% of samples positive for bacterial growth of Acinetobacter, while 84% of samples are negative for growth.

Antibiotic Susceptibility and Detection.of ESBLs

Acinetobacter isolates showed strong resistance to ceftazidime, carbenicillin, azithromycin, and amoxicillin. Ceftriaxone, gentamicin, cefotaxime, nitrofurantoin, amoxicillin-clavulanic acid, naldixic acid, and norfloxacin were the next susceptible antibiotics [9]. The majority of the identified Acinetobacter was discovered to be susceptible to ciprofloxacin, imipenem, amikacin, meropenem, and netilmicin, as shown in the table 1 and figure 2.

The presence of ESBLs enzymes was tested in all isolates of A. baumannii (n = 16). Two (12.5%) isolates were positive for ESBLs as well as were confirmed as multidrug resistance isolates which were resistant to four classes of antimicrobials [10]. Additionlly, were not found in all ESβLs production isolates resistant to all classes of antibiotics, to be considered as extensive or pandrug resistance isolates figure 1.

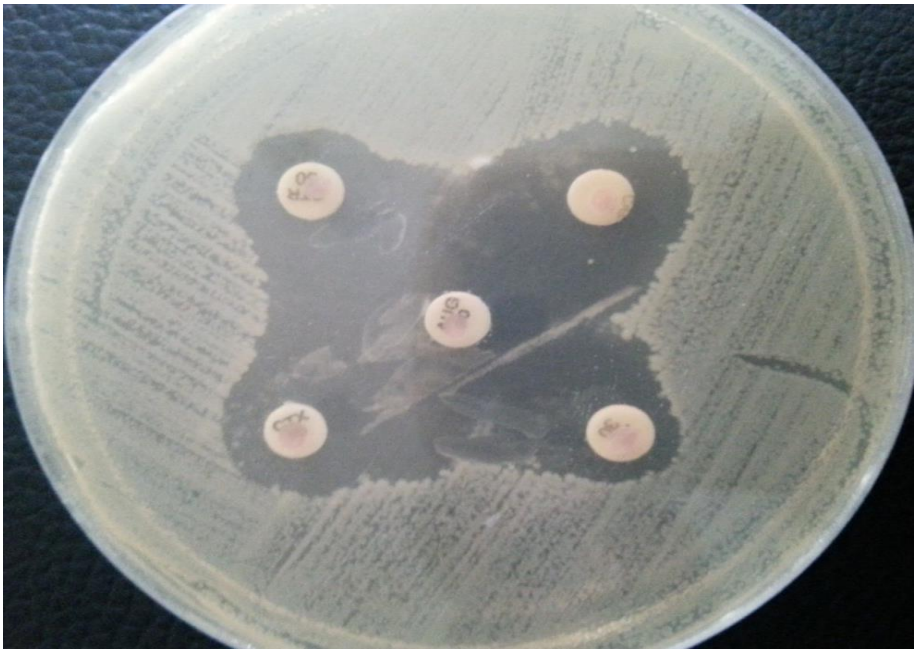

Figure 1. The production of ESBLs is demonstrated through the extension of the inhibition zone between: AUG and CTX; AUG and CAZ, or both.

Table 1. Patterns of antibiotic susceptibility in isolates of Acinetobacter baumannii (n=16)

Antimicrobial	Susceptible(%)	Intermediate(%)	Resistant(%)
Amikacin	8(50)	3(18.75)	**5(31.25)**
Ciprofloxacin	4(25)	2(12.5)	**8(50)**
Imipenem	12(75)	0	**4(25)**
Meropenem	12(75)	0	**4(25)**
Ceftriaxone	1(6.25)	1(6.25)	**14(87.5)**
Gentamicin	2(12.5)	1(6.25)	**13(81.25)**
Cefotaxime	1(6.25)	1(6.25)	**14(87.5)**
Norfloxacin	5(31.25)	1(6.25)	**10(62.5)**
Naldixic acid	4(25)	0	**12(75)**
Netilmicin	12(75)	0	**4(25)**
Carbencillin	2(12.5)	0	**14(87.5)**

Amoxicillin-clavulanic acid	1(6.25)	3(18.75)	**12(75)**
Amoxicillin	0	0	**16(100)**
Ceftazidim	1(6.25)	1(6.25)	**14(87.5)**
Nitrofurantion	1(6.25)	1(6.25)	**14(87.5)**
Azithromycin	1(6.25)	1(6.25)	**14(87.5)**

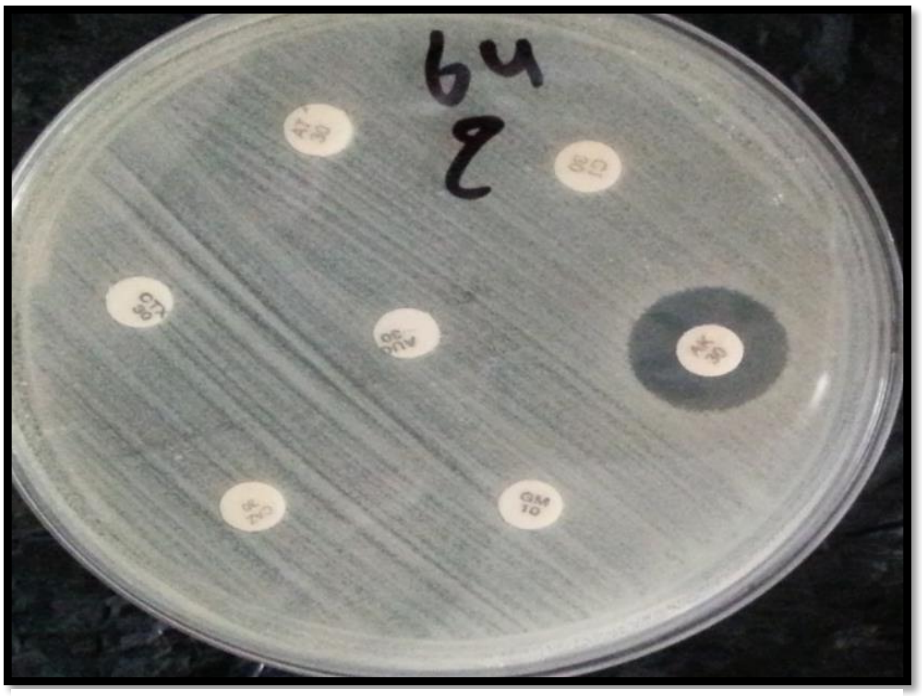

Figure 2. Show the resistance of Acinetobacter baumannii for antibiotics

4. Discussion

In hospitals and the community in general, antimicrobial resistance has grown to be an ongoing medical and public health concern [11]. The patterns of antibiotic susceptibility will therefore be helpful for determining which antibiotic to use and subsequently, reducing hospitalizations, morbidity, and mortality. Reasonable antimicrobial resistance guidelines will benefit from this information. However, due to a shortage of resources, these data or information are rare or unavailable in many of Iraqi provinces; similarly, in Al-Nasiriyah City, the data or information on resistance of antimicrobial among bacterial pathogens is random and inadequate [12].

The prevention and infections treatment that caused by A. baumannii, that makes the ES βLs enzyme, has recently received interest [13]. ES βL-producing strains have been reported from a number of locations across the world, including Iraq, Palestine, Europe, North America, and China [14]. The following factors were involved in the rise in isolate resistance: Long-term hospitalization, the use of last-resort medications, the transfer of susceptible isolates to other patients, susceptible isolates acquired a plasmids encoding antibiotic resistance genes, following this, by way of patient-to-patient transfer, stabilize these resistance isolates [15].

Most ESBLs are generated as a result of mobile genes that are usually coding for resistance against various medications such as aminoglycosides and fluoroquinolones, as well as cephalosporins [16]. ESBLs are known to be present in gram-negative bacteria like Acinetobacter, which are broadly distributed in Iraq [17].

According to Rodrigues et al. (2004), out of 286 Gram-negative isolates, E. coli and Klebsiella were the main producers of ESBLs, then followed by Enterobacter and Acintobacter spp [18]. The analysis of this study shows that the susceptibility of all Acinetobacter isolates tested to various antibiotics indicates that isolates have a high rate of resistance to commonly used antibiotics, as in table 1 explains the antimicrobial susceptibility results [19,20]. According to results of the Al-Shara (2013) study conducted in the city of Najaf, A. baumannii is resistant to both ceftriaxone (51.8%) and ceftazidime (60.5%), two third-generation cephalosporins [21].

Shali (2012) in Sulaimani city show there are multidrug resistance to various antibiotics is present in all isolates. The percentages of resistant were noted for each isolate; the highest rate of resistance was observed against ampicillin (100%) and the lowest rate against imipenem (57.1%) [22]. A. baumannii's cephalosporin resistance in Iraq has been reported by AL-Kadhmi (2015) all A. baumannii isolates were 100% resistant to ceftazidime, cefepime, and cefotaxime. Asaad, et al. (2013) revealed 91% of A. baumannii in Saudi Arabia 2013 were resistant to ceftazidime [23].

5. Conclusion

Conclusion wounds could contain A. baumannii that have ESBL and are resistant to multiple classes of antibiotics; therefore, this needs to be taken into consideration when selecting the appropriate antibiotic to prevent diffusion of such resistant bacteria.

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