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Antimicrobial Resistance Patterns of *Acinetobacter Haemolyticus* from Urinary Tract Infections and Hospital Wastewater

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Keywords: *Acinetobacter haemolyticus*, MIC, Antibiotic Resistance, Hospital Wastewater, Urinary Tract Infection.

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Abstract:

Acinetobacter haemolyticus is a pathogenic species and a bacterium resistant to various types of antibiotics. This study aims to identify the source of *Acinetobacter haemolyticus* infections and determine the most effective treatment options. The research also compares isolates from wastewater and urinary tract infection (UTI) patients to evaluate differences in their antibiotic susceptibility and resistance. Additionally, it examines whether antibiotics effective against clinical UTI isolates show the same effectiveness against wastewater isolates. From thirty samples, 5 samples of urine isolated (33.3%) were of *Acinetobacter haemolyticus*, while the remaining 10 samples (66.6%) showed negative growth. However, out of 15 hospital wastewater samples, 3 (20%) isolated *Acinetobacter haemolyticus*, whereas the remaining 80% showed negative growth. Furthermore. The present results showed that *Acinetobacter haemolyticus* was sensitive to some antibiotics, including ampicillin, gentamicin, piperacillin-tazobactam, cefepime, cefuroxime, trimethoprim-sulfamethoxazole, amikacin, ceftazidime,

cefoperazone-sulbactam, and minocycline, and resistant to two types of antibiotics, including imipenem and meropenem. In this study, even though *Acinetobacter haemolyticus* was isolated from two distinct sources (sewage and urine), it produced identical results in terms of minimum inhibitory concentration, antibiotic resistance, and appropriateness. This work opens the door up to several other investigations. One of those genes examined each type of bacterium based on where it was isolated (inside or outside the human body), revealing the differences between the bacteria's genes and whether or not environmental factors affected these bacteria.

Keywords: *Acinetobacter haemolyticus*, Minimum Inhibitory Concentration, Antibiotic Resistance, Hospital Wastewater, Urinary Tract Infection.

أنماط المقاومة المضادة للميكروبات لبكتيريا *Acinetobacter haemolyticus* المعزولة من التهابات المسالك البولية ومياه الصرف الصحي في المستشفيات

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المستخلص:

تعدُّ البكتيريا الانحلالية نوعا ممرضا ومقاوما لأنواع مختلفة من المضادات الحيوية. تهدف هذه الدراسة إلى تحديد مصدر عدوى البكتيريا الانحلالية وتحديد خيارات العلاج الأكثر فعالية ضدها. فضلا عن ذلك، قارن البحث بين عزلات من مياه الصرف الصحي ومرضى التهاب المسالك البولية لتقييم الاختلافات في حساسيتهم للمضادات الحيوية ومقاومتهم لها. و يدرس البحث ما إذا كانت المضادات الحيوية الفعالة ضد عزلات التهاب المسالك البولية السريرية تُظهر نفس الفعالية ضد عزلات مياه الصرف الصحي. من بين ثلاثين عينة، عُزلت ٥ عينات بول (٣٣,٣٪) من البكتيريا الانحلالية، بينما أظهرت العينات العشر المتبقية (٦٦,٦٪) نموًا سلبيًا. ومع ذلك، من بين ١٥ عينة من مياه الصرف الصحي للمستشفيات، عُزلت ٣ عينات (٢٠٪) من البكتيريا الانحلالية، بينما أظهرت العينات المتبقية (٨٠٪) نموًا سلبيًا. وأظهرت النتائج أن بكتيريا *Acinetobacter haemolyticus* حساسة لبعض المضادات الحيوية، بما في ذلك الأمبيسلين، والجنتاميسين، والبيبراسيلين-تازوباكتام، والسيفيبيم، والسيفوروكسيم، والتريميثوبريم-سلفاميثوكسازول، والأميكاسين، والسيفتازيديم، والسيفوبيرازون-سولباكتام، والمينوسيكلين، ومقاومة لنوعين من المضادات الحيوية، بما في ذلك الإيميبينيم والميروبينيم. وعلى الرغم من عزل بكتيريا *Acinetobacter haemolyticus* من مصدرين مختلفين (مياه الصرف الصحي والبول)، إلا أنها أسفرت عن نتائج متطابقة من حيث الحد الأدنى للتركيز المثبط، ومقاومة المضادات الحيوية، والملاءمة. لذا، يفتح هذا العمل الباب أمام العديد من الأبحاث الأخرى مثل امكانية فحص أحد جينات كل نوع من البكتيريا بناءً على مكان عزله (داخل أو خارج جسم الإنسان)، كاشفًا عن الاختلافات بين جينات البكتيريا وما إذا كانت العوامل البيئية تؤثر عليها أم لا.

الكلمات المفتاحية: البكتيريا الانحلالية، الحد الأدنى من التركيز المثبط ، مقاومة المضادات الحيوية، مياه الصرف الصحي في المستشفيات، عدوى المسالك البولية.

1. Introduction:

Members of the bacterium genus *Acinetobacter* have become one of the most significant drivers of hospital-acquired infections in the past 20 years, overcoming their relative obscurity, demonstrating an amazing capacity of these organisms to acquire antibiotic resistance determinants, with some strains already exhibiting resistance to all clinically used antibiotics [1]. New antibacterial substances are desperately needed to counter the threat posed by *Acinetobacter* species and other uncontrollable bacterial infections. The logical development of antimicrobial medications finds appealing targets in the fundamental mechanisms of transcription, cell division, and chromosomal DNA replication [2]. *Acinetobacter haemolyticus* is a significant species; its resistance to antibiotics has risen due to abuse of antibiotics and the frequent gene exchange between bacteria in hospitals [3]. *Acinetobacter* species are characterized as aerobic, small bacilli that thrive in moist environments easily isolated from various sources like sewage, water, food, and soil [4].

These species are oxidase-negative, catalase-positive, non-motile coccobacilli that thrive in damp environments. Non- fermenting and non-fastidious, the prevalent and clinically significant species of this genus are *Acinetobacter baumannii* and *Acinetobacter haemolyticus* [5]. In intensive care units, *Acinetobacter* species are responsible for about 20% of bacterial infections, making them one of the opportunistic bacteria associated with hospital-acquired infections [6]. *Acinetobacter* species can also be isolated from other sources including pus, wound swabs, sputum, tissue, bronchial washings, cerebrospinal fluid, blood and urine [7]. UTI, ventilator-associated pneumonia, wound infections, and septicemia are among the nosocomial illnesses caused by *Acinetobacter*. The following are risk factors for *Acinetobacter* infections: prolonged hospital stays, immunodeficiency, surgery, burns, aging, the use of antibacterial medications, and invasive devices. Almost all commonly given antimicrobial drugs, such as aminoglycosides, fluoroquinolones, and broad-spectrum beta-lactams, are seeing an increase in resistance in *Acinetobacter* species. Although most strains are resistant to the cephalosporin class of medications, reports of carbapenem resistance are on the rise [8].

Acinetobacter's antibiotic susceptibility patterns might fluctuate greatly between geographical locations and even over time in several hospital units. In order to choose the best course of action, it is crucial to routinely check these infections because of these differences. *Acinetobacter* infections have a major effect on rates of morbidity and death, which raises mortality among critically sick patients. *Acinetobacter* is normally regarded as a low-pathogenicity bacterium but when isolated from individuals who are very sick or have impaired

immune systems, it can be quite dangerous. Usually, nosocomial infections rather than community-acquired illnesses are linked to these pathogens [9].

Acinetobacter spp. accounted for 3.7 and 7.5% of isolates, respectively, making them the eighth and fifth most frequent infections in the USA and Europe, respectively, in a recent series that examined over 13,000 patients hospitalized with pneumonia. Endotracheal intubations, surgery, previous antibiotic treatment, and underlying pulmonary illness are risk factors. The development of a bronchopulmonary fistula, pleural effusion, and multilobular involvement are possible clinical manifestations. According to some reports, mortality rates as high as 70% may be due to the underlying illness of the patient rather than the organism's virulence [10]. The purpose of this research is to determine the origin of *Acinetobacter haemolyticus* infections and to determine the most effective treatment strategy.

2. Material and methods:

2.1. Study Design

Research samples were taken from Tuz Hospital in Sala Hedin Governorate from January to May 2025 from two sources for *Acinetobacter haemolyticus* (urine and hospital wastewater). The sterilization method was used for taking samples without contamination, which were then cultured on necessary media for bacterial growth, diagnosed as *Acinetobacter haemolyticus*, and confirmed by the DL microbial ID/AST system (Enterobact. IST CARD) model DL-120E, and the Minimum Inhibitory Concentration of *Acinetobacter haemolyticus* was detected.

2.2. Collect Samples

The number of samples used for research to identify *Acinetobacter haemolyticus* are 30. 15 samples of urine were collected and placed in sterile urine cups collected from UTI of patients that return to hospital, and other 15 samples of hospital waste water were collected into 250ml sterile screw-cap containers and transported to the Microbiology Laboratory for isolated *Acinetobacter haemolyticus*. After that, they were cultured on MacConkey agar using the streaking culture method, and then incubated for 24 hours at 37°C.

2.3. Isolation and Identification *Acinetobacter haemolyticus*

2.3.1 *Acinetobacter haemolyticus* characteristic

The samples taken from the UTI patients and hospital wastewater (urine and sewage water) were cultured on suitable culture media for growth of *Acinetobacter haemolyticus*, including MacConkey agar [HI Media/India]. The bacteria appeared as colorless to light pink colonies, then cultured on eosin methylene blue agar for differentiation of these bacteria that appeared with blue colonies from other gram-negative bacteria on the MacConkey agar, and all the types

of samples were cultured on sheep blood agar within 48 hours at 37°C. The bacteria growth was found to be 2-3 mm diameter gray-white, convex colonies [11].

2.3.2 Microscopic Identification

A drop of distilled water was added to *Acinetobacter haemolyticus* isolates that had been obtained and placed onto a microscope slide. After that, the smear was inspected under a light microscope after being stained using the Gram stain procedure. Under high magnification and oil immersion, the bacterial cells' color, which indicated the Gram reaction, as well as their form and arrangement, was evaluated [12].

2.3.3. *Acinetobacter haemolyticus* confirmed identification

Acinetobacter haemolyticus identification by DL microbial ID/AST system (Enterobact. IST CARD) model DL-120E and detected Minimum Inhibitory Concentration of *Acinetobacter haemolyticus* [Biotech]. The *Enterobacter* (IST CARD) model DL-120E is used to identify *Acinetobacter haemolyticus*; it depends on the calorimetric characteristic for identification (oxidase test, catalase test, the citrate utilization test, the methyl-red test, the indole test, the Voges-Proskauer test, and the lactose fermentation test, and it depends on turbidity for susceptibility testing to detect minimum inhibitory concentration (CLSI & EUCAST) standard. DL IST card covers nearly 14 types of antibiotics, including ampicillin, gentamicin, ampicillin-sulbactam, piperacillin-tazobactam, cefepime, cefuroxime, trimethoprim-sulfamethoxazole, cefotaxime, imipenem, meropenem, amikacin, ceftazidime, cefoperazone-sulbactam, and minocycline, with multi-level concentration, up to 9 dilutions, for giving true minimum inhibitory concentration value [13].

Methods

1-Add pure *Acinetobacter haemolyticus* colonies to the diluent (0.5 MCF).

2-Add 100 µL of Pure from *Acinetobacter haemolyticus* suspension to biochemical wells (A1 to A12 – B1 to B12) and 50 µL to C1 – C6

3-Add 50 µL of *Acinetobacter haemolyticus* suspension into Muller Hinton broth and mix well

4-Add 50 µL of Muller Hinton broth into antimicrobial wells.

5-Add 2 drops of oil into A1 to A7.

6-Cover the card and place in incubator (18-24 hr.)

7-Add 1 drop of IND and PHE, and add 1 drop of VP1 and VP2 into the corresponding wells.

8-Read the result by D2MINI software.

3. Results and Discussion

3.1. *Acinetobacter haemolyticus* source and number

Between January and May 2025, thirty (30) samples were obtained for the diagnosis of *Acinetobacter hemolyticus*, fifteen from patients who returned to Tuz Hospital with UTI, and fifteen from the hospital's sewage water in the Sala Hedin Governorate. Of these, five urine samples (33.3%) had positive growth for *Acinetobacter haemolyticus*, ten samples (66.6%) had negative growth, three samples (20%) of fifteen wastewater samples had positive growth, and the remaining 80% had negative growth. The DL microbial ID/AST method was used to validate the identification of *Acinetobacter haemolyticus* following bacterial culture and microscopic examinations, as indicated in **Table 1**. An isolate of all *Acinetobacter haemolyticus* was cultivated on MacConkey agar and stained with Gram's stain, which allowed isolates to be recognized based on their morphological traits. It tested negative for Gram stain and coccobacilli, which are classified as strictly aerobic **[14]**.

Table 1: The sample source and number

Sample source	Sample Number	Positive result	Percentage	Negative result	Percentage
UTIs	15	5	33,3%	10	66.6%
Hospital wastewater	15	3	20%	12	80%

Free-living bacteria called *Acinetobacter* species are frequently found in damp environments and are easily separated from sources like soil, food, water, and sewage **[15]**. Catalase-positive, oxidase-negative, tiny, aerobic, non-fermenting, non-fastidious, and non-motile coccobacilli that thrive in damp environments are these species **[16]** Found as various species, like *Acinetobacter lwoffii* and *Acinetobacter haemolyticus*, the species are the most opportunistic non-fermenting bacteria linked to hospital-acquired illnesses, accounting for around 20% of cases **[17]**. Urinary Tract Infections are a considerable global concern and are among the most prevalent types of microbial infection globally **[18]**. Many bacteria can be isolated from urine, such as *Escherichia coli* and *Staphylococcus aureus*, that take part with *Acinetobacter haemolyticus* and cause Urinary Tract Infection **[19, 20]**. Additionally, it can be isolated from numerous species of the genus *Acinetobacter*, which are accountable for nosocomial diseases, such as aspiration pneumonia and catheter-associated infections; they can also cause urinary tract and soft tissue infections. *Acinetobacter haemolyticus* was identified from sewage water. community-acquired pneumonia and bacteremia can occur, especially in hot and humid tropical settings. According to the National Nosocomial Infections Surveillance System, the number of

Acinetobacter infections in the United States increased by 54% between 1987 and 1996, compared to November through June, between July and October (45). High humidity levels are thought to encourage the growth of germs, and water baths and humidifiers have frequently been identified as environmental reservoirs. Additionally, isolate *Acinetobacter haemolyticus* had a proportion of 13 (12%) in this investigation. **[21]**.

3.2 Biochemical properties determination of Minimum inhibition concentration

Acinetobacter haemolyticus was diagnosed using the DL-120E system through a 28-test panel, providing results within 18-24 hours. This approach ensures timeliness and high accuracy (about 99%) and a very low chance of mutation, mitigation, and any risks or errors (**Table2).**

Table2: the results of the DL-120E system.

Test	Result	Test	Result	Test	Result	Test	Result
Glu	-	C	-	ARAF	-	Raff	-
H2s	-	CIT	-	LACI	-	ESC	-
URE	-	MLT	-	SUCF	+	SALf	+
ORN	-	PHE	+	MELF	-	MDGf	+
ARG	-	INO	-	RHAF	-	ONPG	-
LYS	+	VP	-	INOF	+	GEL	-
SORF	-	ADOF	+	MALF	-	CELF	+

All isolates of *Acinetobacter haemolyticus* were subjected to minimum inhibition concentration using fourteen different types of antibiotics, including gentamicin, ampicillin, ampicillin-sulbactam, piperacillin-tazobactam, cefepime, cefuroxime (IV), trimethoprim-sulfamethoxazole, cefotaxime, imipenem, meropenem, amikacin, ceftazidime, cefoperazone-sulbactam, and minocycline. To ascertain the isolates' susceptibility and resistance to the 14 antibiotics. The DL-120E system was used to calculate minimum inhibition concentration. The results are shown in **Table 3 and Figure 1.** The study showed all eight isolates with 100% sensitivity to ampicillin, gentamicin, piperacillin-tazobactam, cefepime, cefuroxime (IV), trimethoprim-sulfamethoxazole, amikacin, ceftazidime, cefoperazone-sulbactam, and minocycline, and with 100% resistance to two types of antibiotics (imipenem and meropenem).

Table3. Minimum Inhibition Concentration (MIC)

Antibiotic	MIC	Resistance (R)	Sensitivities (S)
Ampicillin	≤8\4 ug/ml		S
Gentamicin	≤1ug/ml		S

Ampicillin-Sulbactam	≥16/8ug/ml	intermediate	
Piperacillin-Tazobactam	≤4/4ug/ml		S
Cefepime	≥0.12ug/ml		s
Cefuroxime(vi)	≥4ug/ml		s
Trimethoprim-Sulfamethoxazole	≤0.5/9.5ug/ml		S
Cefotaxime	≥0.12ug/ml		S
Imipenem,	≤0.25ug/ml	R	
Meropenem	≥32ug/ml	R	
Amikacin	≤4ug/ml		s
Ceftazidime	≥32ug/ml		S
Cefoperazone-Sulbactam,	≥8\4ug/ml		s
Minocycline	≥1ug/ml		S

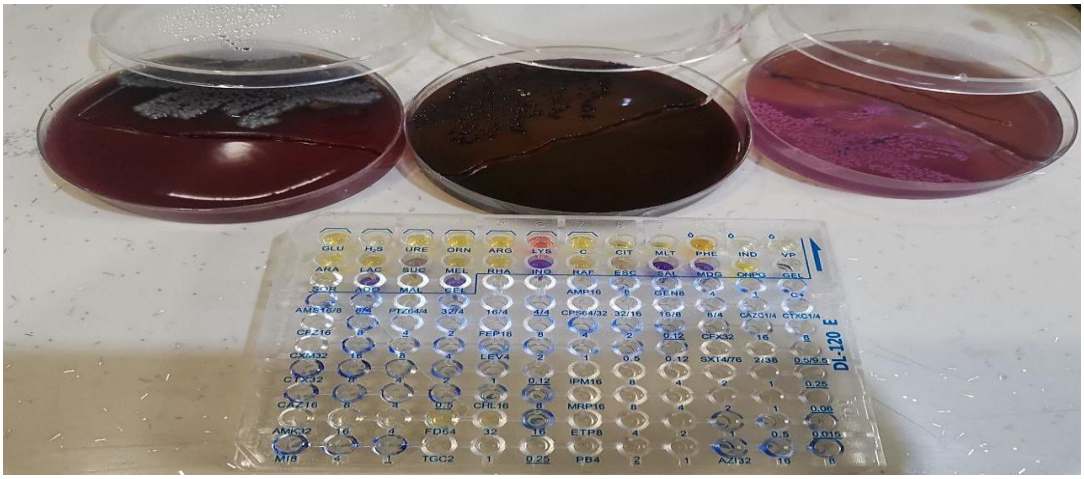


Figure 1: DL-120E system for detected MIC of *Acinetobacter haemolyticus*

A key factor in determining the best course of therapy is minimum inhibition Concentration, which is the lowest concentration of an antimicrobial drug that stops a bacterium from growing visibly. Nevertheless, this approach has a number of shortcomings, particularly with regard to antibiotic resistance. Usually, the minimum inhibition Concentration approach involves giving antibiotic dosages that either inhibit or eradicate the most susceptible bacteria. Even if they are only slightly resistant to the antibiotic, bacteria can still live and proliferate. Treatment failure may result from the growth and dominance of these resistant bacteria over time. Antimicrobial resistance is largely caused by this process, which is referred to as selective pressure [22, 23]. The Bhaduri *et al*. [24] studied *Acinetobacter haemolyticus* isolated in a tertiary care hospital

that isolating *Acinetobacter haemolyticus* in 3.8% and found high levels of resistance to ampicillin-sulbactam (96%), ampicillin (94%), aztreonam (94%), cefuroxime (92%), and ceftazidime (91%). In another study, one important *Acinetobacter* pathogen is *Acinetobacter haemolyticus*, and antibiotic overuse and the frequent gene exchange amongst hospital bacteria are the main causes of *Acinetobacter haemolyticus* resistance. In this investigation, we sequenced the whole genomes of two strains of *Acinetobacter haemolyticus*, TJR01 and TJS01 **[25] [26]**.

Numerous technical aspects impact the pathogenesis and virulence of *Acinetobacter*. The function of lipopolysaccharide or lipopolysaccharide oligosaccharide in these bacteria's outer membranes is of special interest **[27]**. It has been demonstrated that changes to these structures confer resistance to antibiotics and enhance resistance to desiccation. Additionally, it has been shown that *Acinetobacter* species that contain capsules are protected from complement-mediated death. *Acinetobacter's* quivering motility, adherence to environmental surfaces, and biofilm formation are all facilitated by the pellicles that are present on its surface **[28, 29]**.

4. Conclusions

Acinetobacter haemolyticus is an opportunistic pathogen that mainly affects patients with compromised immune systems. In healthy individuals, it is regarded as a low-grade pathogen that they may carry on their skin asymptomatically (colonization). It usually results in infections in hospitalized patients, long hospital stays, and catheters, and the capacity of *Acinetobacter* species, especially *Acinetobacter haemolyticus*, to become resistant to several antibiotics poses a serious threat to human health (multidrug resistance). Therefore, this study highlighted the sources of these opportunistic bacteria. It appeared that even though *Acinetobacter haemolyticus* was isolated from two distinct sources (wastewater and urine), it produced identical results in terms of minimum inhibition concentration, antibiotic resistance, and appropriateness. The goal of this study was accomplished when it was shown that bacteria have the same effect on antibiotic resistance or sensitivity regardless of their origin, and it opens the way up to several other investigations. One of those genes examined each type of bacterium based on where it was isolated (inside or outside the human body), revealing the differences between the bacteria's genes and whether or not environmental factors were affected.

Author Contribution

Jilan Ahmed Shakur: Writing and diagnosis, Alaa R. Nadim: collected samples from UTI patients, Wael Adil Obaid: Writing and desing, Rafeef A.Yass: collected sample from sewage water of hospital.

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Data Availbility Statement

Samples were taken from Toz General Hospital from patients coming to the hospital and from Wastewater of laboratory, which is considered one of the main and suitable sources for the growth of these bacteria.

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Confleicts Of Interest

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Ethical Approval

The local ethical commission at Imam Jaafar AI- Sadiq University, Iraq, approved the undertaking (Approval No.096477651).

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