Multidrug Resistance Profiles of Acinetobacter Baumannii Isolated from Various Clinical Specimens in Duhok City, IRAQ

Original Article

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ABSTRACT

Background: Acinetobacter baumannii is a major health risk and is linked to a high death rate. The current study aimed to determine the antibiotic resistance pattern of A. baumannii from various clinical samples in Duhok City, Iraq. A cross-sectional study was done, and A. baumannii was isolated from several clinical samples from Shyrian and VIN Private Hospitals from September 2022 to November 2023. Identification of bacteria and patterns of antibiotic resistance were carried out according to the Clinical and Laboratory Standards Institute's recommendations. 72 out of 350 A. baumannii were isolated from different clinical samples, including wound scars, sputum, blood, and urine. Most of the isolates, A. baumannii, were isolated as follows: 40.3% were isolated from sputum, 27.8% from surgical wounds, 18.1% from blood, and 13.8% from urine. This study found that antibiogram was significantly resistant among the isolates A. baumannii as follows: the highest resistance was found with Amoxicillin/Clavulanic acid, Cefuroxime, Cefoxitin, Cefixime, Cefotaxime, Ciprofloxacin, Fosfomycin, Nitrofurantoin, Tobramycin and Tetracycline (100.0%), Piperacillin/Tazobactam, Ceftazidme, and Ceftriaxone (91.7%), Amikacin and Cefepime (87.5%), Meropenem (79.2%), Imipenem and Gentamicin (75.0%), Trimethoprim-sulfamethoxazole(50.0%), colistin and Tigecycline (20.8%). A. baumannii was more sensitive to colistin, tigecycline, and trimethoprim/sulfamethoxazole (79.2%, 54.2, and 41.7%), respectively. Finally, the percentage of resistotypes/biotypes of isolated A. baumannii; the most resistant was resistotype 2 (25.0%), and the lowest was resistotype 11 (2.7%). The study found that the frequency of isolation of multiple antibiograms of A. baumannii isolates in Duhok City, Iraq.

Key Words: Acinetobacter baumannii, antibiogram resistance, nosocomial infections.

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INTRODUCTION

Acinetobacter baumannii (A. baumannii) is a Gram-negative Coccobacillus bacterium, a non-motile and non-fermenting bacterium[1,2]. The majority of species under this genus have become prevalent pathogens that can cause infections both in the community and in hospitals[2]. A. baumannii is a major source of nosocomial infections that pose a serious risk to the public's health[3,4]. It is linked to a high death rate and has been identified as an agent of meningitis, pneumonia, septicemia, urinary tract infections, and wound infections. Many virulence factors, such as porins, capsules, cell wall lipopolysaccharide, enzymes, biofilm formation, motility, and iron-acquisition systems, among others, contribute to pathogenesis in A. baumannii infections[3,5]. These virulence factors aid in the organism's ability to withstand harsh environmental circumstances and permit the development of serious diseases[6].

A.baumannii infections are the source of numerous diseases, which has raised serious concerns globally. The most frequent occurrence that facilitates A. baumannii for survival and resistance to most antibiotics is biofilm development, which contaminates medical equipment[7,8]. In addition to developing antibiotic resistance, bacterial biofilms can also become resistant to chemicals, phagocytosis, and other elements of the body's innate and acquired immune systems[9]. This bacterium can spread by contact with hands that come into contact with one another, sputum, urine, faces, and hospital surfaces infected with fomite[10]. Due to A. baumannii's high prevalence of multidrug resistance (MDR) to the majority of commercially

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available antibiotics, it caused serious healthcare issues for patients in ICU wards[11,12]. The current study aimed to isolate A. baumannii from various clinical samples among patients who attended the Vin and Arveen private hospitals in Duhok City, Iraq, and investigate the susceptibility of isolated A. baumannii to different antimicrobials.

MATERIALS AND METHODS

Collection of samples and Identification of A. baumannii

A cross sectional study was conducted from September 2022 to November 2023. The present study was conducted to isolate A. baumannii from different clinical samples. All samples were collected from patients who visited Vin and Arveen Privet hospitals in Duhok City, Iraq. The specimens taken from patients include surgical wounds, blood, sputum, and urine, routinely processed by the Department of Laboratory Service at VIN and Arveen Hospitals. Several identifications and tests of the susceptibility of the isolates were done using the VITEK2 system. The media used are blood agar (5-7% defibrinized blood) and MacConkey agar (Difco, USA). The media were prepared according to the manufacturer's instructions in a 500-mL bottle and sterilized by autoclaving at 121°C for 20 minutes. Then the plates were incubated at 37°C for 18–24 hours in an incubator. Isolated colonies were subjected to Gram staining procedures and the VITEK2 system for identification and antibiotic sensitivity tests on A. baumannii isolates.

Isolation of A. baumannii by VITEK2 System

The VITEK2 system (Biomerieux) is highly automated and uses very compact plastic cards (credit card size) that perform rapid identification based on colorimetry. This system uses repetitive turbidimetric monitoring of bacterial growth during an abbreviated incubation period.

Preparation of suspension

A sterile swab was used to transfer a sufficient number of colonies of pure culture and suspended in 3.0 ml of normal sterile saline (0.45% to 50%) with a PH (4.5–7.0) in a clear plastic polystyrene test tube. The turbidity was adjusted according to the tables provided by the manufacturer's recommendation on the McFarland turbidity range for Gram-positive (0.5-0.63) and measured using a turbidity meter called the DensiChek TM.A test tube containing the bacteria suspension was placed into a special rack (cassette), and the identification card (type VITEK® 2GN ID card for Identification of Gram-Negative Bacteria) was placed in a neighboring slot while inserting the transfer tube into the corresponding suspension tube, and the filled cassette was placed manually after reading the barcode of the cards.

RESULTS

Isolation of A. baumannii from different clinical samples

72 out of 350 A. baumannii were isolated from different clinical samples, including wound scars, sputum, blood, and urine. Most of the isolates, A. baumannii, were obtained from patients who had pneumonia in 29 (40.3%) of sputum samples. Twenty-20 (27.8%) strains were isolated from surgical scar swab cultures, 13 (18.1%) were isolated from blood sample, and 10 (13.8%) from urine, as mentioned in Table 1.

Table 1: The rate of Acinetobacter bumannii isolates from different types of clinical samples.

Types of Samples	Percentage%
Sputum	29 (40.3)
Surgical scar	20 (27.8)
Blood	13 (18.1)
Urine	10 (13.8)
Total	72 (100)

Antibiotic Susceptibility Profile of A.baumannii

Table 2 shows the antibiogram was significantly resistant among the isolates A. baumannii as follows: the highest resistance was found with Amoxicillin/ Clavulanic acid, Cefuroxime, Cefoxitin, Cefixime, Cefotaxime, Ciprofloxacin, Fosfomycin, Nitrofurantoin, Tobramycin and Tetracycline (100.0%), Piperacillin/ Tazobactam, Ceftazidme, and Ceftriaxone was (91.7%), Amikacin and Cefepime (87.5%), Meropenem (79.2%), Imipenem and Gentamicin (75.0%), Trimethoprim-sulfamethoxazole(50.0%), colistin and Tigecycline (20.8%). A. baumannii was more sensitive to colistin, tigecycline, and trimethoprim/sulfamethoxazole (79.2%, 54.2, and 41.7%), respectively.

Table 2: Antibiotic Susceptibility Profile of A.baumannii.

Antibiotics	Sensitive No. (%)	Intermediate No. (%)	Resistant No. (%)
Amoxicillin/Clavulanic acid, Cefuroxime, Cefoxitin, Cefixime, Cefotaxime, Ciprofloxacin, Fosfomycin, Nitrofurantoin, Tobramycin and Tetracycline	0 (0.0)	0 (0.0)	72 (100)
Piperacillin/Tazobactam	6 (8.3)	0 (0.0)	66 (91.7)
Ceftazidme and Ceftriaxone	0 (0.0)	6 (8.3)	66 (91.7)
Amikacin	9 (12.5)	0 (0.0)	63 (87.5)
Cefepime	0 (0.0)	9 (12.5)	63 (87.5)
Meropenem	15 (20.8)	0 (0.0)	57 (79.2)
Imipenem and Gentamicin	18 (25.0)	0 (0.0.)	54 (75.0)
Trimethoprim/sulfamethoxazole	30 (41.7)	6 (8.3)	36 (50,0)
Tigecycline	39 (54.2)	18 (25)	15 (20.8)
Colistin	57 (79.2)	0 (00)	15 (20.8)

Table 3 shows the frequency of resistotypes/biotypes of isolated A. baumannii; the most resistant was resistotype 2 (25.0%), and the lowest one was resistotype 11 (2.7%), and the other resistotypes (1, 3, 4, 5, 6, 7, 8, 9, and 10) were as follows, respectively (16.7%, 12.5%, 9.7%, 4.2%, 4.2%, 4.2%, 6.9%, 8.3%, and 5.6%).

Table 3: Isolated and Percentage of resistotypes and biotypes.

Resistotype patterns	Resistance spectrum phenotypic	Percentage %
Resistotype 1	AMC, CXM, FOX, CTX, CFM, TOB, CIP, TE, FOT, NIF, COL, AK, CXT	12 (16.7)
Resistotype 2	CTX, FOX, CFM, CXM, PTZ, PRL, AMC, CFM, CAZ, CRO, IMP	18 (25.0)
Resistotype 3	FOT, COL, TE, CIP, TOB, GN, AK, MEM, IMP, FEP, CRO, CAZ, CFM, CTX, FOX	9 (12.5)
Resistotype 4	NIF, CIP, AMC, TOB, GN, AK, MEM, IMP, FEP, CRO, CAZ	7(9.7)
Resistotype 5	AMC, CXM, FEP, TOB, AK, GN, CIP, TE, NIF	
Resistotype 6	TIG, PRL, PTZ, CXM, FOX, CTX, AMC, CFM	3 (4.2)
Resistotype 7	CXM, PTZ, PRL, AMC, FOX, CTX, CFM, AMC, CAZ	
Resistotype 8	TOB, GN, AK, IMP, CRO, NIF, FOT, COL, TE, CIP, AMC	5 (6.9)
Resistotype 9	STX, NIF, FOT, CIP, TOB, GN, MEM, IMP, PRL, CXM, FOX, AMC, CAZ, CTX, CFM,	6 (8.3)
Resistotype 10	SXT, NIF, FOT, COL, CIP, GN, AK, MEM, IMP, AMC, CTX, CFM	4 (5.6)
Resistotype 11	NIF, FOT, TE, CIP, GN, AK, MEM, CRO, CAZ, PTZ	2 (2.7)
	Total	72 (100.0)

DISCUSSION

Due to A. baumannii having the ability to produce hospital-acquired infections and treatment failures brought on by numerous antibiotic resistances, it has grown to be a significant health concern[13-15]. Cross-contamination across patients who originate from the same source is demonstrated by the same bacteria that were isolated from various patients at a clinic. In this situation, it is necessary to look into the origin of the microbe that is causing the hospital-acquired infection. The source of the infection, the carrier, and the mode of transmission can all be identified, and appropriate preventive measures selected by determining the clonal link between the isolates[8].

Current study findings reported A. baumannii (72.0%) from various clinical samples, including blood, urine, sputum, and surgical scars. This finding is in agreement with a study done in Erbil Province, Iraq, by ***Sehree et al.*** (2021), who isolated A. baumannii from several clinical samples with a high rate[5]. A similar finding was reported in Nigeria by ***Nwadike et al.***[16] This may be due to the fact that A. baumannii can survive for a long time in a hospital[17]. Also, this result is in line with ***Musyoki et al.***[17], who recorded A. baumannii from different clinical samples at 95.0% and is within the line of Nath and Barkataki,[18] in a study done in India, who reported a high percentage of A. baumannii (30.0%). This study also reported a high prevalence rate of A. baumannii in sputum samples (40.3%), followed by surgical wounds (27.8%). The same result was recorded in two studies done by Antunes and Visca[19] and ***Huang et al.,***[20] who approved that the most predominant cases of A. baumannii were isolated from ICU patients with severe pneumonia. This result is due to A. baumanni, a nosocomial pathogen that can survive and spread, especially in severely ill patients. This is due to its tendency to withstand harsh settings and many classes of antibiotics, which increases morbidity and mortality[21]. Finally, this study observed the low rate of isolation of A. baumannii in blood and urine samples (18.1% and 13.8%), respectively. The same observation was approved by ***Sivaranjani et al.,*** 2013[21] and by ***Sehree et al.***, 2021[22].

It is noticed from the present study results that A. baumannii appeared to be highly resistant to most antimicrobial agents, including: Amoxicillin/Clavulanic acid, Cefuroxime, Cefoxitin, Cefixime, Cefotaxime, Ciprofloxacin, Fosfomycin, Nitrofurantoin, Tobramycin and Tetracycline (100.0%), Piperacillin/Tazobactam, Ceftazidme, and Ceftriaxone was (91.7%), Amikacin and Cefepime (87.5%), Meropenem (79.2%), Imipenem and Gentamicin (75.0%), ***Queenan et al.,***[23] who reported that A. baumannii have resistance for several antibiotics. These findings were documented in Baghdad City, Iraq, by AL-Saleem, 2013[24] and in Tehran City, Iran, by ***Babapour et al.***[9] The current study found that A. baumannii was more sensitive to colistin, tigecycline, and trimethoprim/sulfamethoxazole (79.2%, 54.2, and 41.7%), respectively. These results are supported by Nath and Barkataki, 2016[18] and by ***Sehree et al.,*** 2021[22] The results of this study suggest that colistin, tigecycline, and trimethoprim/sulfamethoxazole were the most effective antibiotics for lowering the A. baumannii infection because Colistin binds to lipopolysaccharides in Gram-negative bacteria's outer membrane, changing the composition of phospholipid bilayers. Through the installation of an osmotic imbalance, this event results in cell death[25].

Finally, the current study revealed that the most prevalent resistotype/biotype of isolated A. baumannii was resistotype 2 (25.0%), the lowest one was resistotype 11 (2.7%), and the other resistotypes (1, 3, 4, 6, 7, 8, 9, and 10) were as follows, respectively (16.7%, 12.5%, 9.7%, 4.2%, 4.2%, 4.2%, 6.9%, 8.3%, and 5.6%). A study was done by ***Ratto et al.,*** 1995; they approved that the most prevalent resistotype is Resistotype 2[26]. While this result disagrees with ***Gonzalez et al.,*** 1998, they said that the most frequent one was resistotype 9[27]. ***Bello et al.,*** 1997, did not support this study and reported that the most prevalent resistotpes were 8 and 9[28, 29].

CONCLUSION

One of the most important factors that contribute to nosocomial infections, especially in intensive care units, is A. baumannii. A. baumannii is highly resistant to most antimicrobial agents, including Amoxicillin/Clavulanic acid, Cefuroxime, Cefoxitin, Cefixime, Cefotaxime, Ciprofloxacin, Fosfomycin, Nitrofurantoin, Tobramycin, and Tetracycline, followed by Piperacillin/Tazobactam, Ceftazidme, Ceftriaxone and Cefepime, Meropenem, Imipenem, and Gentamicin. While this study reported is highly sensitive for colistin, tigecycline, and trimethoprim/sulfamethoxazole high sensitivity, as a result, those promising antibiotics were thought to be a good option for treating A. baumannii multiple antibiotic resistance.

ETHICAL APPROVAL

The study proposal was approved by the ethical and scientific committee at the College of Health Sciences, University of Duhok, Iraq, with code No. 202410.

CONFLICT OF INTERESTS

There is no conflicts of interest.

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AUTHOR CONTRIBUTIONS

The author conceived this work and drafted and finalized this study

DATA AVAILABILITY

The data that support the findings of this study are available on request from the corresponding author.

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أنماط مقاومة الأدوية المتعددة لبكتيريا Acinetobacter baumannii المعزولة من عينات سريرية مختلفة في مدينة دهوك

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الخلفية: تعتبر Acinetobacter baumannii (A. baumannii) من المخاطر الصحية الرئيسية وترتبط بمعدل وفيات مرتفع. تهدف الدراسة الحالية إلى تحديد نمط مقاومة المضادات الحيوية لـ A. baumannii من عينات سريرية مختلفة في مدينة دهوك بالعراق. أجريت دراسة مقطعية، وتم عزل A. baumannii من عدة عينات سريرية من مستشفيات شيرايان و فين الخاصة من سبتمبر ٢٠٢٢ إلى نوفمبر ٢٠٢٣. تم تحديد البكتيريا وأنماط مقاومة المضادات الحيوية وفقًا لتوصيات معهد المعايير السريرية والمخبرية. تم عزل ٧٢ من أصل ٣٥٠ A. baumannii من عينات سريرية مختلفة، بما في ذلك ندبات الجروح والبلغم والدم والبول. تم عزل معظم عزلات A. baumannii على النحو التالي: تم عزل ٤٠,٣٪ من البلغم، و٢٧,٨٪ من الجروح الجراحية، و١٨,١٪ من الدم، و١٣,٨٪ من البول. وجدت هذه الدراسة أن المضادات الحيوية كانت مقاومة بشكل ملحوظ بين عزلات A. baumannii على النحو التالي: تم العثور على أعلى مقاومة مع أموكسيسيلين / حمض كلافولانيك، سيفوروكسيم، سيفوكسيتين، سيفكسيم، سيفوتاكسيم، سيبروفلوكساسين، فوسفوميسين، نيتروفورانتوين، توبراميسين وتتراسيكلين (١٠٠,٠٪)، بايبيرسيلين / تازوباكتام، سيفنازيدمي، وسيفترياكسون (٩١,٧٪)، أميكاسين وسيفبيم (٨٧,٥٪)، ميروبينيم (٧٩,٢٪)، إيميبينيم وجنتاميسين (٧٥,٠٪)، تريميثوبريم-سلفاميثوكسازول (٥٠,٠٪)، كوليستين وتيجيسيكلين (٢٠,٨٪). كانت A. baumannii أكثر حساسية للكوليستين والتيجيسيكلين والتريميثوبريم / سلفاميثوكسازول (٧٩,٢٪ و٥٤,٢ و٤١,٧٪) على التوالي. وأخيرًا، كانت نسبة الأنماط المقاومة / الأنماط الحيوية لعزل A. baumannii؛ الأكثر مقاومة كانت النمط المقاوم ٢ (٢٥,٠٪) وأقلها كانت النمط المقاوم ١١ (٢,٧٪). وجدت الدراسة أن تواتر عزل المضادات الحيوية المتعددة لعزلات (A. baumannii) في مدينة دهوك بالعراق.