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Identification of Antibiotic Resistance of Acinetobacter baumannii in Iraqi Patients

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Abstract

A. baumannii is an opportunistic pathogen that was once regarded as a low-category pathogen but is now a prominent source of hospital and community-acquired illnesses. It is a prevalent cause of pneumonia. To investigate the prevalence of antibiotics resistance among A baumannii.

Two-hundred clinical smaples were collected including (burns, sputum, urine and wound swabs) from individuals suffering from different complaints such as surgical and wound infections, burns inflammation, inflammation of the urinary tract, pneumonia and COVID-19 patients. 86 A. baumannii bacterial isolates could be picked up from these samples using routine test and VITEK-2 system. After diagnosis the A baumannii strains were subjected to an antibiotic sensitivity test using Kirby-Bauer disk diffusion method against Seven type of antibiotics.

A baumannii isolates a exhibited high resistance rate under the effect of tetracycline, Ciprofloxacin, Meropenem and Amikacin antibiotics (92%,76, 72%,72%) respectively. Multidrug resistance profile of 86 A baumannii isolates were 47.6% DR, 37.6% XDR and 15.1 MDR. A significant difference was noticed between the susceptibility profile of 86 A. baumannii isolates at (F.S.P. 50.5, p-value= 0.001).

Reduced susceptibility or resistance to carbapenems is increasingly being observed in A. baumannii clinical isolates, which is an opportunistic bacterium associated with serious hospital infections.

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Keywords: A. baumannii; Amikacin; MDR; Meropenem; Sputum.

1. Introduction

Acinatobacter baumannii an opportunistic pathogen has a high incidence through immunocompromised individuals, especially those who have experienced elongated hospital stay, usually associated with aquatic environments [1]. A.baumannii, A .nosocomialis and A. pittii are the most commonly isolated hospital species [2].

In immunocompromised patients, it is a common cause of pneumonia and septicemia.It had been identified as an ESKAPE pathogen (Enterococcus faecium Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, and Enterobacter species) , a group of pathogens with a high rate of antibiotic resistance that are responsible for the majority of nosocomial infections[3]. On the one hand, it is resistant to several antibiotic classes due to chromosome-mediated genetic factors, and on the other, it can persist in hostile surroundings (walls, surfaces, and medical devices) in hospital settings [4].

There are three types of antibiotic resistance mechanisms of A. baumannii are as follows: first, resistance can be developed by reducing membrane permeability or increasing antibiotic efflux, thereby preventing access to the target. Second, bacteria can protect the antibiotic target through genetic mutation or post-translational modification, and ultimately, antibiotics can be inactivated directly through hydrolysis or modification process [5]. During the last years, this pathogen displayed multidrug resistance (MDR), mainly due to extensive antibiotic abuse and poor stewardship. MDR isolates are associated with medical history of long hospitalization stays, presence of catheters, and mechanical ventilation, while immunocompromised and severely ill hosts predispose to invasive infections [6]. The aims of this study are to describe the susceptibility status of A. baumannii isolated from different clinical samples under different types of antibiotics.

2. Materials and methods

2.1. Specimen collection

This study was accompanied during the period from December 2021 to March 2022. Eighty-six clinical samples were isolated from different sources (burns, sputum, urine and wound swabs) individuals suffer from different complaints such as surgical and wound infections, burns inflammation, inflammation of the urinary tract, pneumonia and, COVID-19 patients in Al-Fallujah Teaching Hospital.

Nomenclature			
C F U	colony forming unit	MDR	multidrug resistant
CLSI	Clinical and Laboratory Standards Institutes	XDR	Extensively drug resistant
ESKAPE	faecium, S. aureus, K. pneumoniae, A. baumannii, P. aeruginosa, and Enterobacter spp.	F.E.P	Fisher ‘s exact probability test
Ib/In2	Pounds per square inch	H S	Highly significant

2.2. Bacterial isolation

The preparation of all culture media was done as specified by the guidelines of the manufacturer; the autoclave at 121ºC (15 Ib/In2) for 15 minutes was used to sterilize media, for primary isolation all the collected clinical samples were streaked on Blood agar, MacConkeys agar. Gram stains and colony morphology characteristics were followed to diagnose the A. baumannii.

2.3. Identification of A. bumannii by VITEK-2 System

The VITEK-2 system was used in this study to confirm the of diagnosis of A. baumannii isolates, which included several steps according to [7] .Preparation of bacterial suspension: By using a sterile swab, a suitable number of A. baumannii colonies from a pure culture were transferred and separately suspended in 3 ml of sterile saline in a clear plastic test tube, with the goal to adjust the turbidity of bacteria to 0.5 MacFarland using BioMerieux \DensiCHEK plus (France) , which equals to (1.5*108 C F U/ml).

2.4. Antibiotics Sensitivity Test

Kirby-Bauer disk diffusion method was followed and the turbidity of A. baumannii bacteria to 0.5 MacFarland as mentioned above were subjected. After adjusting the turbidity of bacteria carpet method was used for spreading the bacteria over Muller hinton agar. Seven type of antibiotic (Bioanalyse- Turkey) were applied including Impeneam (10mg), Tetracycline (10mg), Meropenem, (10mg), Levofaxean (5mg), Amikacian (30mg), Ciftraxone,(30mg) and Polymyxin-B (300).

2.5. Statistical analysis

The Chi-square (X2) test was used to compare percentages in the current study's data. The test was conducted using a significance level of 0.05. To analyze current data, SPSS v.23 programs were utilized [8].

3. Results and Discussion

3.1. Identification of A. baumannii

The results showed that 35 (40.7%) and 34 (39.5%) of A. baumannii bacterial isolates could be picked up from burn 64 (32%) and sputum 70 (35%) out of 200 clinical samples as arranged in Table 1. Acinetobacter baumannii has rapidly emerged as a prominent opportunistic pathogen and substantial causes of morbidity and mortality in healthcare settings in recent years. The ability of the organism to survive in a hospital environment for an extended period of time, develop antibiotic resistance quickly, and propagate clonally all contribute to the species' persistence and transmission in healthcare settings. As a result, A. baumannii has the potential to cause a wide range of serious nosocomial infections, including ventilator-associated pneumonia, wound infections, bloodstream infections, meningitis, and urinary tract infections. Debilitating patients, those admitted to intensive care units (ICUs), those with indwelling foreign devices, and those who are mechanically ventilated are among the high-risk categories [9, 10].
The current results were comparable to [12] which is a local study that concluded there were 96 cases of infection of multi-drug resistant, extensive-drug resistant and pan-drug resistant A. baumannii were 79.65% in sputum, 10.62% in urine, 4.42% in wound secretion, 3.54% in blood, and 1.77% in other drainage discharges, respectively. While the increase of A. baumannii in wounds due to its ability to biofilm formation in the skin this result was in agree with [13]. Who mentioned the increase of A. baumannii in wounds due to its ability to biofilm formation in the skin and soft tissue infections, as well as in occlusive dressings.

Table 1 Distribution A. baumannii (N=86) according clinical samples

Samples	N (%)	A. baumannii (N, %)
Burn	64 (32)	34 (39.5)
Sputum	70 (35)	35 (40.7)
Urine	14 (7)	8 (9.3)
Wound	35 (17.5)	8 (9.3)
Bed sore	8 (4)	0 (0)
Blood	9 (4.5)	1(1.2)
Total (N, %)	200 (100%)	86 (100%)

3.2. Bacterial isolation

Because of the rise in hospital-acquired infections and the growth of treatment resistance, doctors face a huge problem due to infection by A. baumannii (12).Results declared that (86) isolates were diagnosed as A. baumannii by growth on blood agar they seemed opaque creamy and non-hemolytic colonies, convex, gray or white color [14], While MacConkey agar media were identified gram negative coccobacilli their

colonies seemed small, pale yellow and lactose non fermenter [15], who mention that isolates obtained from MacConkey agar media were identified according to the Gram stain, A. baumannii showed gram negative coccobacilli and arranged in diplococci Fig. 1.

Fig 1. Colony morphology of A. baumannii on MacConkey agar

3.1. Distribution of Acinetobacter. bumannii According to Type of Specimen

In this study, a (86) A. baumannii isolated from different specimens distributed according to the specimens type. There were (35) isolates from sputum with high percentage 40.7 %, from burns with percentage 33(38.4%), from the wound isolate 9(10.5%) , also from urine 8 (9.3 %) ,and the low percent isolate from blood 1(1.2%), significant difference was noticed at p-value = 0.001 (H.S) F.E.P=50.8, Fig. 2. shows the percent of A. baumannii distribution according to specimens.

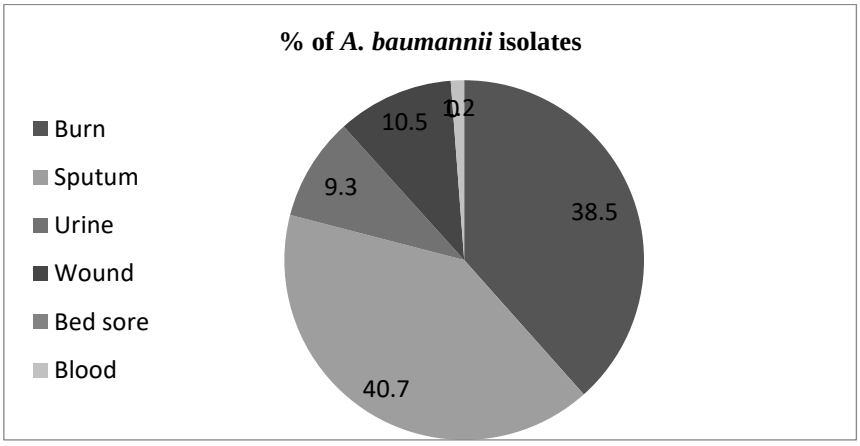
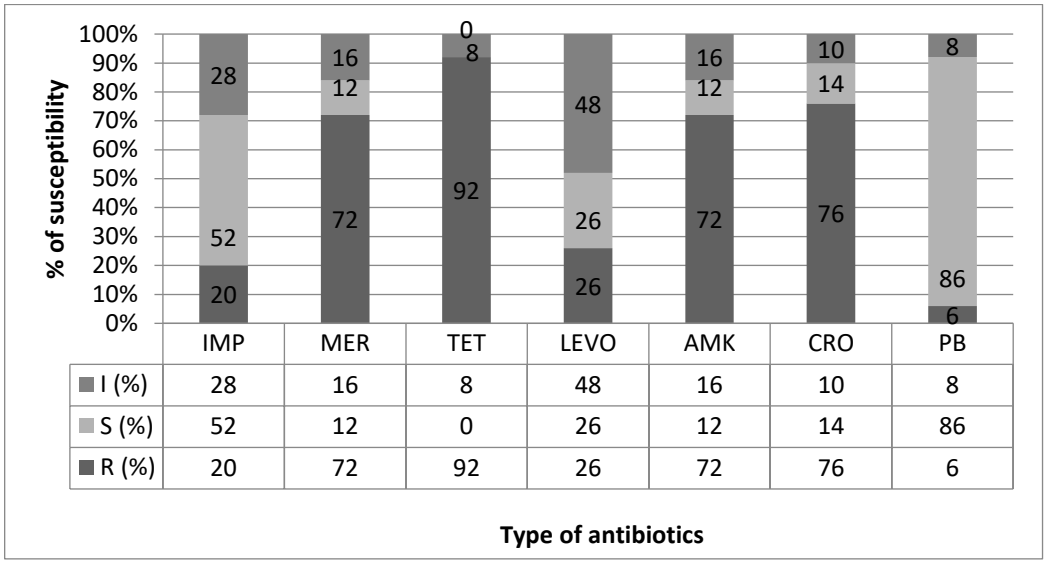


Fig 2. Distribution of A. baumannii according to type of specimen

3.2. Distribution of A. baumanii infection according to antibiotic resistance

Globally, the most important factors, involved in raising the rates of antibiotic resistance are the frequent and bad use of antimicrobial agents [16]. Additionally, the improper prescription of the antimicrobial agent due to inadequate surveillance systems for microbiological approaches also contributes to the same target mentioned above [17]. Antimicrobial susceptibility tests were done for all A. baumannii isolates (86 isolates) by using the disk diffusion method (Kirby-Bauer Method) against (7) different clinically important antimicrobials (Imipenem, Meropenem, Amikacin, Tetracycline, Polymyxin-B, Ceftriaxone and Levofloxacin). These isolates showed different susceptibility toward antimicrobials used in this study. The susceptibility to different antimicrobials was determined depending on CLSI (2018) [18], see Figs. 3 & 4.



	IMP	MER	TET	LEVO	AMK	CRO	PB
I (%)	28	16	8	48	16	10	8
S (%)	52	12	0	26	12	14	86
R (%)	20	72	92	26	72	76	6

Fig 3. Distribution of A. baumanii infection according to antibiotic resistance

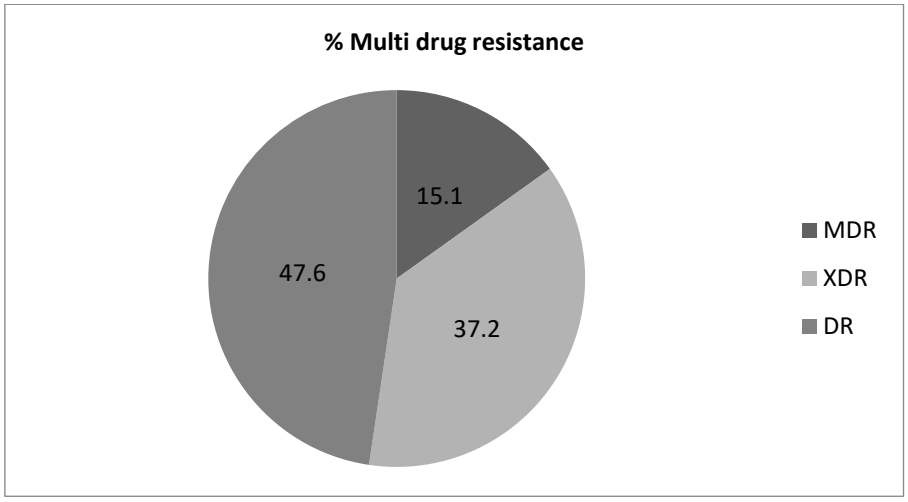


Fig 4. Multi drug resistance profile of A. baumannii isolates (N=86)

5. Conclusions

In summary A. baumannii bacterial isolates were mostly isolated from sputum and burn samples which indicated the ability of A. baumannii bacteria to grow inside the hospitals and contaminate exposed and open tissues. They also contaminated the devices inserted into the respiratory system and acquired and spread the antibiotic resistance. The A. baumannii bacterial isolates were mostly resistant to meropenem antibiotics which are the last choices for treatment of gram negative and gram-positive bacteria which make the treatment of this type of infection very difficult and put the infected patients under severe status.

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Ethical Approval

Ethical approval for this study was granted from the ethical committee of the Iraqi Ministry of Health AL-Anbar Directorate of Health (no. 6125).

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