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EVALUATION OF ANTIBIOTIC SENSITIVITY IN *ACINTOBACTER BAUMANNII* ISOLATES FROM CLINICAL SPECIMENS IN SOME HOSPITALS IN BAGHDAD

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ABSTRACT
The current study aimed to determine antibiotic sensitivity in *Acintobacter baumannii* isolates from clinical specimens in some hospitals in Baghdad. The results of antibiotic sensitivity of eight isolates of *Acinetobacter baumannii* bacteria were chosen for the antibiotics, Ampicillin / Sulbactam, Piperacillin / Tazobactam, Cefazolin, Meropenem, Amikacin, Ceftazidime, Gentamicin, Ceftriaxone, Tobramycin, Cefepime, Ciprofloxacin, Aztreonam, Levofloxacin, Ertapenem, Tigecycline, Imipenem and Trimethoprim / Sulfamethoxazole. Among eight *A. baumannii* isolates evaluated. For some *A. baumannii*, no growth terminations were observed among the eight isolates tested.

KEYWORDS: Antibiotic, Sensitivity, *Acinetobacter baumannii*, clinical specimens.

INTRODUCTION
Acinetobacter is Gram-negative coccobacilli, aerobic, glucose non-fermenting, non-fastidious, non-motile, catalase-positive, oxidase negative bacteria, and negative for indole and nitrate (Lee *et al.*, 2011). The members of the genus *Acinetobacter* are short, plump rods about 1µm in diameter, and 1.5-2 µm in length in the active growth logarithmic phase, and appear coccobacilli during the stationary phase (Bitrian *et al*., 2013).

Acinetobacter spp. can cause infections in both hospital settings and in community. They are the second most commonly isolated non-fermenters in human specimens, after *Pseudomonas aeruginosa*. About 1-3% of health care-associated infections are caused by *Acinetobacter* spp., *Acinetobacter* has little risk to healthy people. However, people with weak immune system, chronic lung disease and diabetes may be more susceptible to infections with *Acinetobacter* (Kempf and Rolain, 2012).

Infection caused by *Acintobacter baumannii* are formidable threats because of their ability to survive on inanimate objects and the remarkable emergence of Multi drug resistant (MDR) and pan-drug resistant (PDR) strains, these infections are difficult to treat owing to innate and acquired antimicrobial resistance (Moutefour *et al.*, 2008).

Multiple bacterial virulence factors are required for pathogenesis of infections caused by *A. baumannii*; these include outer membrane proteins, gelatinase activity, biofilm production, capsular polysaccharides, bacterial phospholipases, penicillin-binding proteins, secreted outer membrane vesicles and siderophores (Park *et al.*, 2012).The first nosocomial outbreak with carbapenam resistant was reported from the united states in 1991. Since then, these infections and extensive hospital outbreaks have been reported throughout the world (Liu *et al.*, 2012). In addition, this organism has been identified in outbreaks of severe infections of wounded military personnel returning in Middle East (Calhoun *et al.*, 2008).

Acinetobacter baumannii outbreaks of infections have been reported worldwide. Colonization or infection with multidrug-resistant *Acinetobacter* is associated with the following risk factors: prolonged hospital stay, admission to an intensive care unit, mechanical ventilation (Bearers *et al*., 2009), and exposure to broad spectrum antibiotics, recent surgery, invasive procedures, and severity of the underlying disease (Maragakis *et al*., 2008).

MATERIALS AND METHODS
Sample collection
This study was conducted during the period from December 2019 to April 2020, During the study period, eighty clinical burn wounds swabs samples were taken. Patients were interviewed and they answered several questions regarding personal information. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from Ghazi Al Hariri Hospital

(40 samples) and Baghdad hospital (40 samples) in Baghdad / Iraq.

Culture media used for the detection of *Acintobacter baumannii*

Nutrient agar

Nutrient agar is a general purpose medium support the growth of wide variety of microorganisms, this media consist of 0.5% peptone, 0.3% beef /yeast extract, 1.5% agar, 0.5% sodium chloride, and distilled water, the PH of this medium is neutral. These components were combined and boiled for one minute for mixing and then sterilized by autoclaving for 15 minutes, after cooling, covering and stored upside down and stored in the refrigerator until used (Jawtez, *et al*., 2010).

Blood agar

Blood agar which considered one of enriched medium used for support the growth of fastidious microorganisms, this medium consist of the same components of Nutrient agar plus 5% sheep blood, the PH of this medium approximately (7.4). Preparation of this medium was done by addition of 28g of Nutrient agar powder in one liter of distilled water, heat the mixture, autoclaving for sterilization, then allow it to cool but not solidified, then 5% of blood was added and mix well, and dispense into sterile plates, finally stored in the refrigerator until used (Jawtez, *et al*., 2010).

MacConkey agar

MacConkey agar considered one of the important selective medium used in bacteriological laboratory, it's usually used for detection of Gram-negative bacteria, it consist of 17g of peptone, 3g of protease peptone, 10g of lactose, 1.5g of bile salts, 5g of sodium chloride, 0.03g of neutral red, 0.001g of crystal violet, 13.5g of agar, and distilled water, the PH of the medium is neutral.

The crystal violet and bile salts inhibit the growth of Gram-positive bacteria, and allow the growth of Gram-negative bacteria, the medium is prepared by mixing the ingredients and boiling, autoclaving, cooling, and stored until used (Jawtez *et al*., 2010).

Brain heart infusion agar

Brain heart infusion is a growth medium for growing microorganisms. It is a nutrient-rich medium, and can therefore be used to culture a variety of fastidious organisms. The broth is often used in food safety, water safety, and antibiotic sensitivity tests.

Brain heart infusion agar typically contains infusion of beef or pig heart as well as calf brain, a source of amino acids (often either digested gelatin or other animal tissue), salt, disodium phosphate as a buffer, and glucose as a source of sugar. Many formulations for this agar also exist, in which agar is added as a gelling agent for growing plates of microorganisms (Atlas, 1995).

Chrmoagar media

One of the important selective media used to identify *Acinetobacter baumannii* as the colonies of this bacteria will appear red in color, while other gram negative bacteria either inhibited or appear blue in color, gram positive bacteria will be inhibited on this media. This medium consist of agar agar, peptone and yeast extract, salts, and chromogenic mix. Direct streaking on the media and incubate for 24 hours at 37°C (Moran-Gilad *et al*., 2014).

Biochemical test used to detect *Acintobacter baumannii*

Biochemical test

Biochemical test as Oxidase, Indole, Methyl red, Lactose fermentation, Citrate utilization, Catalase production and Urease test. were performed on the isolates to confirm their identification *Acintobacter baumannii*. All the tests were according to (MacFaddin, 2000 ; Forbes *et al*., 2007),

API (Analytical Profile Index) and Identification Using Vitek 2 System

Antibiotic sensitivity test used to detect *Acintobacter baumannii*

Isolates were tested for antibiotic susceptibility, using the automated VITEK 2 Compact system. All samples were cultured on blood agar, and then, a suspension was made for every isolate. Liquid suspension of all isolates was loaded on the VITEK system and left overnight to get the results. The next day, the results have illustrated the identication and antibiotic susceptibility of loaded samples. VITEK 2 system was used for authenticating names of Acinetobacter species as described by the manufacturer (bioMerieux Inc., Durham, NC 27712, USA). The VITEK card contains 64 wells, which hold deferent fluorescent biochemical assays. Out of the 64, 20 were carbohydrate assimilation; 4 were phosphatase, urea, nitrate, and actidione tests. The VITEK 2 machine controlled the card automatically including the filling, sealing, and then transferring the cards into the linked incubator (35°C). Each output report is decoded according to a particular algorithmic system. Acquired results were compared to the ID-GN (identication of Gram-negative bacteria) databank. In case of most known Acinetobacter species with a clear cut profile, the system led to a correct identication of the unknown organism.

RESULTS AND DISCUSSION

Study Samples

This study was conducted during the period from December 2019 to April 2020, During the study period, eighty clinical burn wounds swabs samples were taken. Patients were interviewed and they answered several questions regarding personal information. Burn wounds samples from a total of 80 clinical different Wounds, samples were collected from Ghazi Al Hariri Hospital (40 samples) and Baghdad hospital (40 samples) in Baghdad / Iraq. Table (3-1) wounds samples were

collected from different age groups and gender during the period from December 2019 to April 2020. Seventy (87.5%) were clinical wounds positive samples, while the rest (10) were negative wounds samples (12.5 %).

Table (1): Total number of burn wounds samples used for the isolation of bacteria

Clinical wounds samples	Positive (growth)	Negative (no growth)
80	70	10
Percentage	87.5%	12.5 %

At least 25% of healthy individuals may carry *Acinetobacters* as part of their normal skin flora, but carriage of *Acinetobacter* spp. By healthy subjects at other body site is normally low, in contrast, high colonization rate of the throat, skin, respiratory tract or digestive tract of hospitalized patients with clinically significant strains have been reported during outbreaks of infection (Towner, 2006).

Acinetobacter spp. are gram-negative, aerobic, coccobacilli that are ubiquitous in nature, persistent in the hospital environment, and cause a variety of opportunistic nosocomial infections (Bergogne-Berezin and Towner, 1996). The majority of infections caused by *A. baumannii* are contracted in hospitals, most often in critically ill patients hospitalized in intensive care or surgery (McConnell *et al.*, 2012).

Isolation of *Acinetobacter baumannii*

A total of 80 samples were collected from patient suffering from burn wounds. All samples were cultured on selective medium chromagar *Acinetobacter*, blood, nutrient agar and MacConkey agar under 37 ºC for 24 hr to isolates *Acinetobacter* sp.

Identification of bacterial isolates

Several morphological, microscopical and biochemical tests were made to identify bacterial isolates, including culturing on selective medium chromagar, morphological and microscopical characteristics and temperature tolerance of isolates, Then, the characteristics of isolates were identified by biochemical test and confirmation of identification by Analytical Profile Index Api 20 and Vitek 2 Compact system.

Morphological and Microscopical characteristics

From 70 samples inoculated on the Chromagar *Acinetobacter*, 10 isolate grew on the medium, *A. baumannii* isolates on the Chromagar were appeared as bright red colonies after 24h and incubation at 37° C. The identification of *A. baumannii* isolates were depended on culturing these isolates on CHROM agar *Acinetobacter*, MacConkey, blood and nutrient agar (table 2). Characteristics *Acinetobacter baumannii* colonies were after incubation for 24 hrs at 37°C on MacConkey, blood and nutrient agar, on MacConkey agar, colonies of A. *baumannii* appeared as a non lactose fermenter, on blood and nutrient agar, colonies of all isolates were appeared as non pigmented, domed, and mucoid, small, flat colorless, circular. Isolates were stained by gram staining method, their cells appeared under the microscope as gram negative.

Table (2): Number of isolates of *Acinetobacter baumannii.*

Isolate No.	Isolates Name	isolation source
1	AS-1	Ghazi Al Hariri Hospital
2	AS-2	Ghazi Al Hariri Hospital
3	AS-3	Ghazi Al Hariri Hospital
4	AS-4	Ghazi Al Hariri Hospital
5	AS-5	Baghdad Hospital
6	AS-6	Baghdad Hospital
7	AS-7	Baghdad Hospital
8	AS-8	Baghdad Hospital
9	AS-9	Baghdad Hospital
10	AS-10	Baghdad Hospital

CHROMagar Acinetobacter is a recently developed medium for selective and rapid identification of *Acinetobacter* spp. It contains agents that inhibit the growth of most yeasts and gram-positive cocci and uses a color-change method that permits identification of *Acinetobacter* spp. as red colonies within 18-24 hr of incubation. (Gordon and Wareham, 2009).

Acinetobacter baumannii is rod-shaped which grows well on MacConkey agar (without salt). Although officially classified as not lactose-fermenting, they are often partially lactose-fermenting when grown on MacConkey agar (Constantiniu *et al.*, 2004). Growth and purity of cultures of *Acinetobacter baumannii* were determined by culture on MacConkey agar and Blood agar. On MacConkey agar it's formed pale coloured, Non lactose fermenting colonies and on Blood Agar it's

formed non-hemolytic colonies. There was only one type of colonies attesting to its purity (Shrikant, 2017).

Biochemical characteristics

The suspected all isolates of *A. baumannii* and were then subjected to the related biochemical tests. All isolates of *A. baumannii* were found to be catalase positive and oxidase / indole negative. Tests on Lactose fermentation and motility test gave negative results. The positive results for the test appeared in methyl red and Citrate utilization, finally we used the urease test which give negative results for *Acintobacter baumannii*. The isolates were identified as *A. baumannii* and have been confirmed by using Api 20 and VITEK 2 Compact, table (3).

Acinetobacter was identified as non motile, oxidase negative and catalase positive. (Cisneros *et al.,* 2002). *Acinetobacter baumannii* is positive for citrate. Citrate in simmon citrate medium is important to detect weather the bacteria isolates able to grow on it as a unique carbon and energy source. (Macfaddin, 2000).

Non-lactose fermenting and non-motile of *Acinetobacter baumannii* isolates (Hussein *et al.,* 2013). Urease test was positive for *Acinitobacter baumannii* urease enzyme catalyzes the breakdown of urea, and the bacteria that can produce this enzyme is able to detoxify the waste products and to drive metabolic energy from its utilization which change the medium color from yellow to purple-pink, indicating urease positive test. (Atlas *et al.*,1995; Forbes *et al*., 2002).

Acinetobacter baumannii isolates were oxidase negative and catalase positive (Doughari *et al.*, 2011). API NE 20 test VITEK 2 Compact were carried out confirms the biochemical tests regarding the species identification of the *A.baumannii* isolates.

Table 3: Biochemical characteristics of Acinetobacter baumannii.

No	Biochemical Test	Result
1	Oxidase	-
2	Indole	-
3	Motility	-
4	Methyl red	+
5	Lactose fermentation	-
6	Citrate utilization	+
7	Catalase production	+
8	Urease test	+

Demographical picture and clinical presentation

The results of this study were based on the analysis of 30 patients with burn wounds give positive results of *Acintobacter baumannii*, compared with 30 apparently healthy considered as controls. Mean age of patients was 31.86±8.52 years with a range of (18-45) years, while mean age of control group was 32.43±7.08 years with a range of (20-45) years; statistically there was no significant difference in mean age between both groups, this result is a basic requirement to conduct such a case control study (P>0.05), as shown in table (4) of both study groups.

Table 4: Mean age and distribution of patients and apparently healthy subjects according to 10 years age intervals.

Age interval	Patients group		Apparently healthy subjects		P-value
	No.	%	No.	%	
≤ 20 yrs.	4	13.33	1	3.33	
21-30 yrs.	10	33.33	10	33.33	
31-40 yrs.	10	33.33	14	46.67	
> 40 yrs.	6	20	5	16.67	
Total	30	100.0	30	100.0	
Mean age ±SD*	31.86±8.52		32.43±7.08		0.780
Age range	(18-45) year		(20-45) year		

*SD, Standard deviation of Mean

Burn wounds may happened to anybody during his life, this infection disease classify into four stages according to American fertility society, in the current study the highest incidence was the minimal stage I 17 (57.67%) follow with mild stage II 9 (30%) as in figure (1).

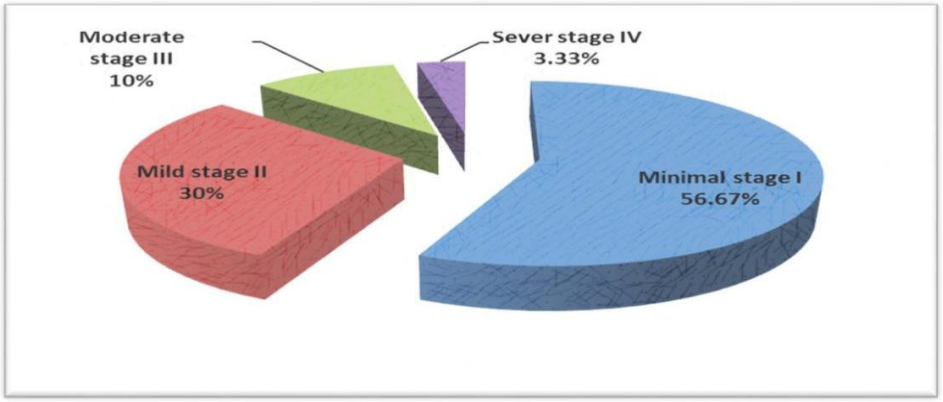

Figure (1): Stages of burn wounds in patients group.

According to familial history, 4 (13.33%) out of 30 patients infected with *Acintobacter baumannii* have family history as shown in table (5).

Table (5): Distribution the patients according the family history.

Family history	Patients		Controls	
	Number	Percent	Number	Percent
No	26	86.67%	30	100%
Yes	4	13.33%	0	0%
Total	30	100%	30	100%

Sensitivity test of *Acinetobacter baumannii* for antibiotics by Vitek 2

The results of antibiotic sensitivity show in table (6) and (7). The Sensitivity of eight isolates of *Acinetobacter baumannii* bacteria were chosen for the antibiotics, Ampicillin/ Sulbactam, Piperacillin/ Tazobactam, Cefazolin, Meropenem, Amikacin, Ceftazidime, Gentamicin, Ceftriaxone, Tobramycin, Cefepime, Ciprofloxacin, Aztreonam, Levofloxacin, Ertapenem, Tigecycline, Imipenem and Trimethoprim / Sulfamethoxazole. Among eight *A. baumannii* isolates evaluated. For some *A. baumannii*, no growth terminations were observed among the eight isolates tested. The emergence of prevalence of resistance is considered as a major therapeutic problem that could be explained by several hypothesis such as, the influence of excessive (Sotto *et al.*, 2007). As the antibiotic becomes more widely used and becomes more prevalent in the market as soon as antibiotic resistance increases. (Risan *et al.*, 2017; Subhi *et al.*, 2017; Risan *et al.*, 2019; Shareef and Risan 2021). Several systems, including the VITEK 2 system, incorporate expert systems to control the results of susceptibility tests by applying a series of predefined rules. This VITEK system monitors the kinetics of bacterial growth and calculates MICs using a unique algorithm. In addition, the VITEK 2 system incorporates several technical improvements which automate many procedures that were performed manually with the previous VITEK system.

Joyanes *et al.*, (2001) shown that VITEK 2 is a new automatic system for the identification and susceptibility testing of the most clinically important bacteria. In the present study 198 clinical isolates, including *Pseudomonas aeruginosa* (n = 146), *Acinetobacter baumannii* (n = 25), and *Stenotrophomonas maltophilia* (n = 27) were evaluated. Reference susceptibility testing of cefepime, cefotaxime, ceftazidime, ciprofloxacin, gentamicin, imipenem, meropenem, piperacillin, tobramycin, levofloxacin (only for *P. aeruginosa*), co-trimoxazole (only for *S. maltophilia*), and ampicillin-sulbactam and tetracycline (only for *A. baumannii*) was performed by microdilution (NCCLS guidelines). The VITEK 2 system correctly identified 91.6, 100, and 76% of *P. aeruginosa*, *S. maltophilia*, and *A. baumannii* isolates, respectively, within 3 h. The respective percentages of essential agreement (to within 1 twofold dilution) for *P. aeruginosa* and *A. baumannii* were 89.0 and 88.0% (cefepime), 91.1 and 100% (cefotaxime), 95.2 and 96.0% (ceftazidime), 98.6 and 100% (ciprofloxacin), 88.4 and 100% (gentamicin), 87.0 and 92.0% (imipenem), 85.0 and 88.0% (meropenem), 84.2 and 96.0% (piperacillin), and 97.3 and 80% (tobramycin).

The essential agreement for levofloxacin against *P. aeruginosa* was 86.3%. The percentages of essential agreement for ampicillin-sulbactam and tetracycline against *A. baumannii* were 88.0 and 100%, respectively. Very major errors for *P. aeruginosa* (resistant by the reference method, susceptible with the VITEK 2 system [resistant to susceptible]) were noted for cefepime (0.7%), cefotaxime (0.7%), gentamicin (0.7%), imipenem (1.4%), levofloxacin (2.7%), and piperacillin (2.7%) and, for one strain of *A. baumannii*, for imipenem. Major errors (susceptible to resistant) were noted only for *P. aeruginosa* and cefepime (2.0%), ceftazidime (0.7%), and piperacillin (3.4%).

Minor errors ranged from 0.0% for piperacillin to 22.6% for cefotaxime against *P. aeruginosa* and from 0.0% for piperacillin and ciprofloxacin to 20.0% for cefepime against *A. baumannii*. The VITEK 2 system provided co-trimoxazole MICs only for *S. maltophilia*; no very major or major errors were obtained for co-trimoxazole against this species. It is concluded that the VITEK 2 system allows the rapid identification of *S. maltophilia* and most *P. aeruginosa* and *A. baumannii* isolates. The VITEK 2 system can perform reliable susceptibility testing of many of the antimicrobial agents used against *P. aeruginosa* and *A. baumannii*. It would be desirable if new versions of the VITEK 2 software were able to determine MICs and the corresponding clinical categories of agents active against *S. maltophilia*.

In study (Bansal, *et al.*, 2020), the antibiotic susceptibility of the *A. baumannii* strains, sixteen antibiotics representing eight antimicrobial classes were used. The antimicrobial agents included tobramycin (18%), ampicillin/sulbactam (32%), gentamicin (32%), cefotaxime (36%), ceftriaxone (36%), cefepime (36%), ceftazidime (43%), tetracycline (43%), trimethoprim / sulfamethoxazole (50%), levofloxacin (54%), imipenem (46%), doripenem (46%), ciprofloxacin (57%), meropenem (57%), and amikacin (61%), respectively. All isolates were sensitive to tigecycline except isolates C4 and C24. Of the 28 isolates, 18% (n = 5) were multidrug-resistant (MDR) and 29% (n = 8) were extreme drug-resistant (XDR) based on criteria provided by Magiorakos and colleagues (Magiorakos *et al.*, 2012).

Ugrakli *et al.*, (2017) found Antibiotics with the lowest resistance to *Acinetobacter* strains were colistin (2 %) and tigecycline (6 %), and a comparatively lower resistance was found when amikacin and gentamicin were compared with other antibiotics.

Table 6: Antibiotic sensitivity test on *Acinetobacter baumannii* isolates.

N.	Antibiotic	No. of isolates	S	R
1	Ampicillin/ Sulbactam	8	1	7
2	Piperacillin/Tazobactam	8	0	8
3	Cefazolin	8	0	8
4	Ceftazidime	8	8	0
5	Ceftriaxone	8	3	5
6	Cefepime	8	6	2
7	Aztreonam	8	0	8
8	Ertapenem	8	0	8
9	Imipenem	8	8	0
10	Meropenem	8	8	0
11	Amikacin	8	8	0
12	Gentamicin	8	8	0
13	Tobramycin	8	8	0
14	Ciprofloxacin	8	8	0
15	Levofloxacin	8	8	0
16	Tigecycline	8	7	1
17	Trimethoprim/Sulfamethoxazole	8	6	2

R: resistant S: susceptible

Table 7: Antibiotic sensitivity test on *Acinetobacter baumannii* isolates.

Isolates	Antibiotic															
	1	2	3	4	5	6	7	8	9	11	12	13	14	15	16	17
AS-1	R	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S
AS-2	R	R	R	S	R	R	R	R	S	S	S	S	S	S	S	S
AS-4	R	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S
AS-5	S	R	R	S	R	S	R	R	S	S	S	S	S	S	S	R
AS-6	R	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S
AS-7	R	R	R	S	S	S	R	R	S	S	S	S	S	S	S	S
AS-8	R	R	R	S	R	R	R	R	S	S	S	S	S	S	R	R
AS-10	R	R	R	S	R	S	R	R	S	S	S	S	S	S	S	S

S = sensitive ; R = Resistance

1-Ampicillin / Sulbactam, 2- Piperacillin / Tazobactam, 3-Cefazolin, 4- Ceftazidime, 5- Ceftriaxone, 6- Cefepime, 7- Aztreonam, 8- Ertapenem, 9- Imipenem, 10- Meropenem, 11- Amikacin, 12- Gentamicin, 13- Tobramycin, 14- Ciprofloxacin, 15- Levofloxacin, 16- Tigecycline, 17- Trimethoprim / Sulfamethoxazole

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