Imipenem-Resistant *Acinetobacter baumannii* isolated from patients and hospitals environment in Baghdad

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Abstract

During 2011, 1900 clinical specimens and 240 hospital environment specimens were collected from four hospitals in Baghdad. 128 isolates of *Acinetobacter baumannii* were obtained from clinical and environmental specimens in a percentage of 6.05% and 5.42%, respectively. The highest percentage of isolation, 38.26% was of sputum specimens and lower percentage of burns specimens 5.22%. The lowest incidence was of age range (71-80) years old group whereas the highest incidence was of age range (31-40) years old group. Also we found that the incidence was higher in males (66.96%) than that of females (33.04%) and the frequency of positive *A. baumannii* isolates was higher in intensive care units (ICUs**)**. Results revealed eleven different resistotype patterns* designated arbitrarily from A-K and all our isolates showed multidrug resistance to those antibiotics. We found the highest percentage (85%) of imipenem resistant *A. baumannii* (IRAB) isolates were isolated from blood disease (leukemia) department followed by ICU, RCU, Burns, surgical and other departments, respectively.

Keywords: Imipenem, *Acinetobacter baumannii*, nosocomial

بكتريا *Acinetobacter baumannii* المقاومة للأمبينيم المعزولة من مرضى و بيئة مستشفيات بغداد

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الخلاصة:

خلال عام 2011 ، 1900 عينة سريرية و240 عينة من بيئة المستشفيات تم جمعها من مستشفيات في بغداد ،تم الحصول على 128 عزلة تعود الى بكتريا *A. baumannii* وبنسبة 6.05% من العينات السريرية و 5.42% من العينات البيئية. أعلى نسبة عزل لهذا النوع وصلت الى 26.38% تم الحصول عليها من نماذج القشع بينما اقل نسبة عزل تم الحصول عليها من نماذج الحروق ووصلت الى 5.22% من 115 عزلة سريرية. كانت أقل نسبة أصابات بين مجموعة الأعمار 71–80 سنة بينما سجلت أعلى نسبة أصابات بين مجموعة الأعمار 31–40 سنة وكانت نسبة الأصابة في الذكور (66.96%) أعلى منها في الإناث (33.04%) و أعلى تكرار للأصابة ببكتريا *A. baumannii* كان في وحدات العناية المركزة. أظهرت النتائج 11 نمطا للمقاومة سميت من A–K وكانت جميع العزلات مقاومة للعديد من المضادات الحياتية ،فيما يخص العزلات السريرية (115) كانت جميع العزلات متعددة المقاومة. تم تسجيل أعلى نسبة عزل وصلت الى (85%) للعزلات المقاومة لمضاد الأمبنيم من ردهات امراض الدم تلاها وحدات العناية المركزة، وحدات العناية التنفسية، ردهات الحروق، الردهات الجراحية والردهات الأخرى.

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1. Introduction

Acinetobacter baumannii is a glucose non-fermentative Gram-negative coccobacillus bacterium [1,2], nosocomial infections has become an increasingly prevalent cause especially in immunocompromised and in Intensive Care Units (ICUs) patients in the last few years [3,4]. *A. baumannii* represents the most clinically important and frequently detected *Acinetobacter* species [5]. During the last two decades, *A. baumannii* has become a pathogen of increased clinical importance due to its remarkable ability to cause outbreaks and its ability to acquire resistance to almost all available antibiotics, including the carbapenems (such as imipenem and meropenem)[6,7].

Carbapenems are the drugs of choice for the treatment of serious nosocomial infections caused by *A. baumannii* [8,9]. Carbapenem-resistant *A. baumannii* strains have been now emerged around the world [10-12].

Furthermore, no study has been performed in Iraq to investigate the distribution of Imipenem-Resistant *Acinetobacter baumannii* in Iraqi patients and hospital environment. Therefore, this study was aimed to investigate the distribution of imipenem-resistant *A. baumannii* in patients and environment of four hospitals in Baghdad and to determine the drug resistance patterns of *A. baumannii* strains isolated from inpatients and hospital environment.

2. Materials and Methods

Specimens collection

All samples were collected from January toDecember 2011, it was about 1900 specimens comprising; urine, wounds, burns, blood and sputum, collected from residing in four hospitals in Baghdad/ Medical city including: Baghdad Teaching Hospital, The Martyr Gazi Al-Hariry Hospital, Welfare Teaching Hospital and The Burn Specialist Hospital.

Also 240 specimens were collected from hospital environment specimens were collected from (patients' beds, tables, sinks, floors, air samples and medical equipments).

Isolation and identification of *Acinetobacter baumannii*

In the laboratory under aseptic conditions, the collected specimens were streaked directly on blood agar and MacConkey agar, incubated for 24 hrs at 37ºC. The non hemolytic opaque creamy colonies on blood agar and non lactose fermenting colonies on MacConkey agar were subcultured on MacConkey agar and incubated for another 24 hrs at 37ºC [13]. All bacterial isolates were examined for gram stainability and conventional biochemical tests which include: Oxidase test, Catalase test, Kligler iron agar (KIA), Indole production test, Motility test, Urease production test, Citrate utilization test, Lactose fermentation test, Hemolysin production, Growth at 44ºC according to [13] and discoloration of blood agar containing D-glucose test[14]. Identification results were confirmed by API 20E system.

Furthermore, species identification of *Acinetobacter baumannii* isolates was performed by using Polymerase chain reaction (PCR) to detect *bla*OXA-51-Like genes [15,16]. DNA of each isolate was extracted using a commercial purification system (Genomic DNA Mini Kit (Geneaid, Thailand)). Forward and reverse primer pair that detecting 353 bp fragments of OXA-51-like genes, were chosen according to the method described by [16,17], and the sequences of primer pair used were OXA-51-Like-F (5'-TAATGCTTTGATCG GCCTTG-3') and OXA-51-Like-R (5'-TGGATTGCAC TTCATCTTGG-3'). Primers were purchased from (Alpha DNA, Canada) as lyophilized form, dissolved in sterile deionized distilled water to give a final concentration of 100 picomole/µl as recommended by provider and stored in a deep freezer until use.

The extracted DNA, primers and PCR premix (Accupower, Bionear (Korea)) that contains: Taq DNA polymerase, $MgCl_2$, deoxynucleotides dNTPs, KCl, stabilizer and tracking dye and Tris-HCl (pH 9.0), was thawed at 4°C, vortexed and centrifuged briefly to bring the contents to the bottom of the tubes. Optimization PCR was accomplished after several trials, PCR mixture was set up in a total volume of 50 µl included 5µl of PCR premix, 2 µl of each primer (10 picomole/ µl) and 4 µl of template DNA (100 ng/µl). The rest volume was completed with sterile D.W. Negative control contained all material except DNA, were D.W. was added instead of template DNA. PCR reaction tubes were vortexed and finally placed into thermocycler PCR instrument (Multigene(Gradient), USA).

The program that used in the thermocyler PCR was carried out according to [16,17], which include ; Initial denaturation at 94°C for 5min followed by30 cycles of (Denaturation at94°C for 25sec, Annealing at 53°C for 40sec and Extension at 72°C for 50sec) then Final extension at 72°C for 6min.

Antibiotic susceptibility test

Kirby-Bauer method was followed as described by [18] to carry out the antibiotics susceptibility test for 20 different antibiotics: amikacin (30µg), amoxicillin-clavulanicacid (20/10µg), aztreonam (30µg), cefalothin (30µg), cefepime (30µg), cefotaxime (30µg), ceftazidime (30µg), ceftriaxon (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), Colistin (10µg), gentamicin (10µg), imipenem (10µg), meropenem (10µg),piperacillin (100µg), rifampin (5µg), tetracycline (30µg), ticarcillin-clavulanate(75/10µg), tobramycin (10µg) and trimethoprime-sulphamethoxazole (1.25/23.75µg). Inhibition zones developed around the discs were measured by millimeter (mm) using a metric ruler according to Clinical Laboratories Standards Institute (CLSI) [19]. *Escherichia coli* (*E. coli* ATCC 25922) was used as a quality control in susceptibility determination.

Statistical analysis

Chi-square (χ^2) test was employed for comparison among groups, t-test was used to analyse other data. P value < 0.05 was considered statistically significant.

3. Results and Discussion

Isolation and identification of *Acinetobacter baumannii* isolates

All isolates appeared as Gram-negative coccobacilli and occasionally arranged in diplococci. All isolates showed negative results for oxidase test, motility test, indole production test and urease production test, while the isolates gave positive results to catalase test and citrate utilization test. Kligler iron agar developed an alkaline slant, no change bottom, H_2S negative without gas production. Also when *A. baumannii* isolates were cultured on MacConkey agar, they appeared as small, pale and lactose non fermenter colonies, while on blood agar they appeared as opaque, creamy and non-hemolytic colonies. Growth at 44ºC was positive for all *A. baumannii* isolates which showed the ability to grow at this temperature degree. This test was used to distinguished *A. baumannii* (which wasable to grow at this temperature degree) from other *Acinetobacter* species which unable to grow at this temperature degree [20,21]. The results of biochemical tests were listed in table 1.

Table 1- Biochemical test results for *Acinetobacter baumannii*.

Id	Biochemical test	Result
1	Catalase production	+
2	Citrate utilization	+
3	Growth at 44ºC	+
4	Hemolysin production	⁻ (γ hemolysis)
5	Indole production	_
6	Lactose fermentation	–
7	Motility	_
8	Oxidase production	_
9	Kliglar iron agar (KIA)	Alkaline slant / No change bottom, No gas , No H_2S
10	Urease production	_

+; positive result, - ; negative result

Isolates of *A. baumannii* were also identified by discoloration of blood agar containing D-glucose, in which all isolates of *A. baumannii* gave a positive result to this test by production of a unique light-brown discoloration of the surrounding blood agar (browning effect), while another two isolates (*Pseudomonas aeruginosa* and *Moraxella catarrhalis*) did not cause similar discoloration (the browning effect was not observed) as it depicted in figure 1.

Figure 2 shows 1900 clinical specimens, 115 (6.05%) were identified as *A. baumannii*. The environmental isolates of *A. baumannii* were diagnosed with clinical isolates and out of 240 hospital environmental samples, 13 (5.42%) were belonge to *A. baumannii*.

Figure 1- Blood agar containing D-glucose after 24 hours of incubation at 37ºC. (A) *Pseudomonas aeruginosa* (B) *Acinetobacter baumannii* (C) *Moraxella catarrhalis.*

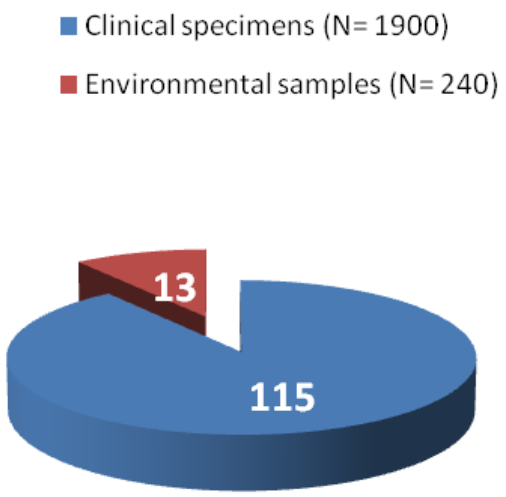

Figure 2- Numbers of Acinetobacter baumannii isolates

4. Distribution of *Acinetobacter baumannii* according to type of specimens

According to table 2, out of 1900 clinical specimens of urine, wounds, burns, blood and sputum, 115 were positive to *A. baumannii* which was isolated in high percentage;38.26% (n=44) from sputum specimens; while, blood specimens constituted 26.09% (n= 30), wounds specimens achieved 23.48% (n= 27), urine specimens formed 6.95% (n= 8) and low percentage was in burns specimens which accomplished 5.22% (n= 6)(P<0.05).

Out of 116 clinically isolated *A. baumannii*, the highest isolation percentage (53.49%) was from respiratory secretions followed by 21.55%, 15.17%, 3.45%, 3.45% and 2.59% from blood, wounds, cerebrospinal fluid, body fluids and urine, respectively [22] . Another study found that about 36 *A. baumannii* isolates 26 (72.2%) *A. baumannii* isolates were from the respiratory tract infections[23] .

Table 2- Number and percentage of *Acinetobacter baumannii* isolates in accordance to specimens type.

Type of specimens	Total number of specimens	No. of positive *A. baumannii* Isolates	Percentage of *A. baumannii* Isolates*
Blood	557	30	9.26
Burns	112	6	5.22
Sputum	498	44	26.38
Urine	365	8	.956
Wounds	358	27	48.23
Total	1900	115	100

***Out of total number of positive *A. baumannii* specimens (n= 115). P<0.05**

5. The distribution of age and sex among infected patients with *Acinetobacter baumannii*

The age and sex distribution of the infected patients was summarized in table 3. Among the infected patients of age ranged from less than 1 month to 80 years, the lowest incidence was among (71-80) years old age group (3.48%), whereas the highest incidence was among (31-40) years old age group (25.22%). The table also shows that the incidence was higher (P<0.05) among males (66.96%) than that of females (33.04%).

A study showed that *A. baumannii* isolates were recovered from 75 patients; 67 (89%) men and 8(11%) women, found also the mean age of those patients was 35 years, with rang from 5 to 86 years [24].

Table 3- Distribution of *Acinetobacter baumannii* according to age and gender

Age groups (year)	Infected patients with *Acinetobacter baumannii*					
	Male		female		Total	
	No.	(%)*	No.	(%)*	No.	(%)*
<10	9	7.83	3	2.61	12	10.43
11-20	5	4.35	2	1.74	7	6.09
21-30	9	7.83	8	6.96	17	14.78
31-40	23	20	6	5.22	29	25.22
41-50	12	10.43	9	7.83	21	18.26
51-60	9	7.83	7	6.09	16	13.91
61-70	8	6.96	1	0.87	9	7.83
71-80	2	1.74	2	1.74	4	3.48
Total	77	66.96	38	33.04	115	100

***Out of total number of positive *A. baumannii* specimens (115). P<0.05**

6. Frequency of positive *Acinetobacter baumannii* isolates according to hospitals departments

Table 4 shows that the frequency of positive *A. baumannii* isolates was higher in Intensive care unit (ICU); 25.22% (n=29) followed by Respiratory care unit (RCU); 18.26% (n=21), leukemia department; 17.39% (n=20), surgical department; 13.04% (n=15), Burns department; 7.83% (n=9) and finally 18.26% (n=21) from all other departments of hospitals (P<0.05).

Table 4- Frequency of positive *A. baumannii* isolates according to hospitals departments.

Hospitals department	Number of positive isolates	Percentage of positive isolates*
Intensive care unit (ICU)	29	25.22
Respiratory care unit (RCU)	21	.2618
Blood disease (Leukemia)	20	17.39
Surgical	15	13.04
Burns	9	7.83
Others	21	18.26
Total	115	100

***Out of total number of positive *A. baumannii* specimens (n= 115).P<0.05**

6. Identification of *Acinetobacter baumannii* by Polymerase Chain Reaction (PCR)

*bla*OXA-51-like genes were found to be present in all 128 (100%) *A. baumannii* clinical and environmental studied isolates. The results of the presence of *bla*OXA-51-like genes are exemplified by the isolates shown in figure 3.

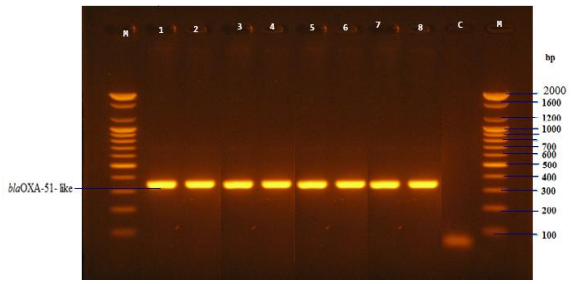

Figure 3- Detection of*bla*OXA-51-Like byPCR. **Lane M,** 100 bp DNA ladder; **lanes 1-8,**different *Acinetobacter baumannii* isolates; **lane C,** Negative control (had all PCR mixture including water instead of DNA template). Detection was done on agarose gel (1.5%) at 5 V/cm for 1.5 hour, stained with ethidium bromide and visualized on a UV transiluminator documentation system.

7. Antibiotics Susceptibility

Data presented in figure 4 shows a high level resistance of *A. baumannii* clinical isolates to most of the antibiotics under test. The present study revealed that all *A. baumannii* clinical isolates had 100% resistance to amoxicillin-clavulanic acid, cefepime, cefotaxime and rifampin. This study also showed a highest resistance to aztreonam (97.39%), ceftriaxone (97.39%), ticarcillin-clavulanate (96.52%), chloramphenicol (95.65%), piperacillin (91.30%), cefalothin(91.03%), ceftazidime (89.57%), gentamicin (87.83%), trimethoprime-sulphamethoxazole (86.09%), ciprofloxacin (83.48%), amikacin (72.17%) and colistin (66.96%). Tobramycin and tetracycline recorded moderate resistance; 46.09% and 47.83%, respectively.

Given to notice that from 115 clinical isolates of *A. baumannii*, 67 isolates (58.26%) were resistant to both imipenem and meropenem and 8 (6.96%) of those isolates were intermediate to both imipenem and meropenem, while 40 (34.78%) of those isolates were sensitive to both imipenem and meropenem (figure 4).

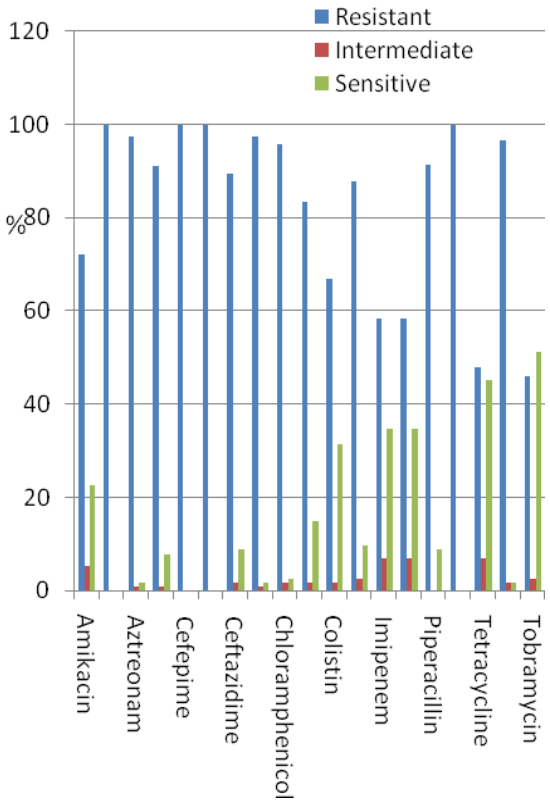

Figure 4- Antibiotic resistance of 115 *Acinetobacter baumannii* clinical isolates.

Similar study carried out in 2006 found that the clinically identified *A. baumannii* isolates were completely sensitive to imipenem while they were 100% resistant to ciprofloxacin, aztreonam and cephalothin. Also, they found that the resistance percentage to tobramycin and tetracycline were 82.35% and 100%, respectively[25] . These differences may be

attributed to the irrespective use of these two antibiotics in our hospitals in the last few years. Another study carried out in 2007 reported that *A. baumannii* clinical isolates showed 100% sensitivity to meropenem [26]. Results of another study carried out in 2010 found that *A. baumannii* clinical isolates developed 100% resistance to cefotaxime, ceftazidime, ceftriaxone, 95.45% to cefepime, chloramphenicol, aztronam and 40.90% to imipenem [27]. Upon these local studies, we can notice interestingly the increase of resistance to imipenem antibiotic in our hospitals.

Analysis of antibiotic resistance patterns showed that all the 115 clinical isolates of *A. baumannii* were multidrug resistant isolates. Such resistance attributed to extensive use of antimicrobial chemotherapy in clinical environments [28,29].

Regarding colistin, our results showed a low level of sensitivity reached to 31.30%. However, many studies reported that the effective antibiotic used to treat imipenem- resistant *A. baumannii* isolate was colistin. For instance, high sensitivity percentages to this antibiotic (100%) were reported by [30-32].

Although the differences were insignificant ($P<0.05$), the results presented in table 5 demonstrate the highest percentage (85%) of imipenem resistant *A. baumannii* (IRAB) isolates were isolated from blood disease (leukemia) department followed by 75.86%, 66.67%, 60%, 44.44% and 42.86% from ICU, RCU, surgical, Burns and other departments, respectively (isolates showing intermediate levels of susceptibility were considered as resistant) [19,20].

Data presented in table 6 demonstrate the highest percentage (77.27%) of imipenem resistant *A. baumannii* (IRAB) isolates were isolated from **s**putum specimens followed by 74.07%, 56.67%, 37.5% and 16.67% from wounds, blood, urine and burns specimens, respectively (isolates showing intermediate levels of susceptibility were classified as resistant) [19,20].

Table 5- Frequency of imipenem resistant *Acinetobacter baumannii* isolates according to hospitals departments.

Percentage of imipenem resistant isolates	Number of imipenem resistant isolates	Total number of *A. baumannii* isolates	Hospitals departments
75.86	22	29	**Intensive care unit (ICU)**
66.67	14	21	**Respiratory care unit (RCU)**
85	17	20	**Blood disease (Leukemia)**
60	9	15	**Surgical**
44.44	4	9	**Burns**
42.86	9	21	**Others**
65.22	75	115	**Total**

Table 6- Frequency of imipenem resistant *Acinetobacter baumannii* isolates according to type of specimens.

Percentage of imipenem resistant isolates	Number of imipenem resistant isolates	Total number of *A. baumannii* isolates	Type of specimens
56.67	17	30	**Blood**
16.67	1	6	**Burns**
77.27	34	44	**Sputum**
37.5	3	8	**Urine**
74.07	20	27	**Wounds**
65.22	75	115	**Total**

On the other hand, the susceptibility of the environment isolates toward different antibiotics can be seen in figure -5, in which the highest resistance percentages (100%) were found to amoxicillin-clavulanic acid, cefepime, cefotaxime, rifampin and ticarcillin-clavulanate. Moreover, 84.62%, 84.62%, 84.62%,76.92% ,76.92% , 69.23%, 69.23%, 61.54%, 61.54% and 53.85% of the environmental *A. baumannii* isolates were resistant to ceftriaxone, chloramphenicol, piperacillin,gentamicin, trimethoprime-sulphamethoxazole, cefalothin, ceftazidime, aztreonam, colistin and ciprofloxacin, respectively. The lowest resistance percentage (7.69%) was detected to (imipenem and meropenem) then (15.38%) to tetracycline followed by (23.08%) and (30.77%) to tobramycin and amikacin, respectively.

The percentage of imipenem resistant *A. baumannii* isolates among environmental isolates (7.69%) was less than among *A. baumannii* clinical isolates (58.26%).

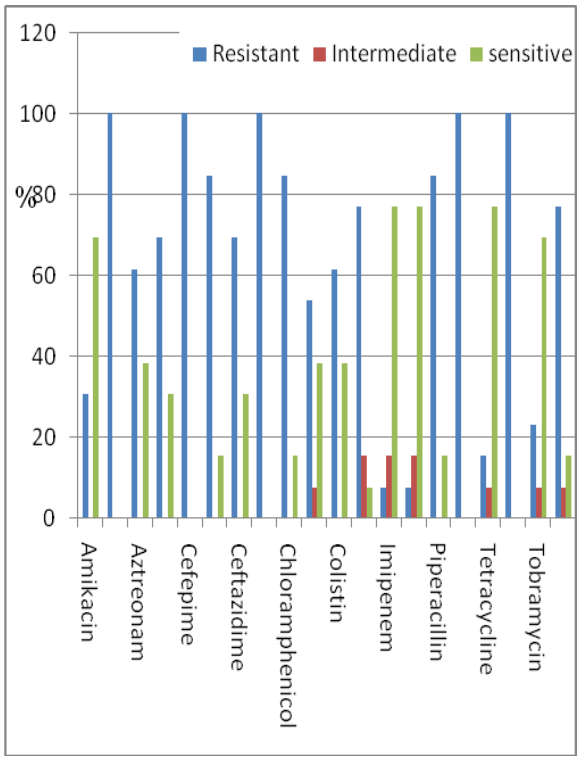

Figure 5- Antibiotic resistance of 13 *Acinetobacter baumannii* environmental isolates.

Resistance of *A. baumannii* clinical and environmental isolates to the tested antibiotics revealed eleven different resistotype patterns designated arbitrarily from A-K resistotypes (isolates showing intermediate levels of susceptibility were classified as resistant) [19,20] as in table -7.

Pattern **A** accounted for 22 *A. baumannii* isolates that were resistant to all antibiotics. Pattern **B** included 15 *A. baumannii* isolates that were resistant to most antibiotics but sensitive to tobramycin only. Pattern **C** comprised 13 *A. baumannii* isolates that were resistant to most antibiotic but sensitive to colistin only. Fifteen *A. baumannii* isolates showed Pattern **D**, being resistant to all antibiotics but sensitive to tobramycin and tetracycline only. Pattern **E** involved 9 *A. baumannii* isolates that were resistant to most antibiotics but sensitive to tetracycline and colistin only. Pattern **F** covered 4 *A. baumannii* isolates that were resistant to most antibiotics but sensitive to tetracycline, tobramycin, amikacin and colistin. Eleven *A. baumannii* isolates showed Pattern **G**, being resistant to all antibiotics but sensitive to imipenem, meropenem, amikacin and colistin. Four *A. baumannii* isolates showed Pattern **H**, being resistant to all antibiotics but sensitive to tobramycin, imipenem, meropenem and gentamicin. Twelve *A. baumannii* isolates showed Pattern **I**, being resistant to all antibiotics but sensitive to tobramycin, tetracycline, imipenem and meropenem . Pattern **J** accounted for 6 *A. baumannii* isolates that were resistant to most antibiotics but sensitive to tetracycline, piperacillin, ciprofloxacin, amikacin, imipenem and meropenem. Finally, 17 *A. baumannii* isolates showed Pattern **K**, being resistant to all antibiotics but sensitive to tobramycin, tetracycline, imipenem, meropenem, amikacin, ciprofloxacin, ceftazidime, cefalothin and pipracillin.

Table 7- Resistotype patterns of *Acinetobacer baumannii* isolates

Pattern	Isolates codes	Description
A	N20, N22, N26, N28, N30, N31, N33, N34, N43, N63, N68, N95, N101, N102, N104, N114, N116, N117, N118, N119, N122, N123	resistant to all antibiotics
B	N29, N36, N64, N70, N85, N86, N89, N92, N96, N98, N106, N107, N110, N111, N115	resistant to most antibiotics but sensitive to tobramycin only
C	N4, N5, N6, N8, N9, N35, N38, N39, N40, N48, N50, N51, N52	resistant to most antibiotics but sensitive to colistin only
D	N32, N62, N65, N69, N79, N83, N84, N93, N97, N99, N105, N120, N121, N124, N125	resistant to all antibiotics but sensitive to tobramycin and tetracycline only
E	N25, N27, N41, N42, N47,N53, N56, N71, N91	resistant to most antibiotics but sensitive to tetracycline and colistin
F	N45, N54, N76, N94	resistant to most antibiotics but sensitive to tetracycline, tobramycin, amikacin and colistin
G	N1, N3, N10, N17, N18, N19, N21, N24, N37, N44, N103	resistant to all antibiotics but sensitive to imipenem, meropenem, amikacin and colistin

H	N11, N15, N59, N126	resistant to all antibiotics but sensitive to tobramycin, imipenem, meropenem and gentamicin
I	N57, N66, N67, N73, N74, N75, N77, N80, N81, N82, N87, N109	resistant to all antibiotics but sensitive to tobramycin, tetracycline, imipenem and meropenem
J	N46, N49, N55,N58, N72, N78	resistant to most antibiotics but sensitive to tetracycline,piperacillin, ciprofloxacin, amikacin,imipenem and meropenem
K	N2, N7, N12, N13, , N14, N16, N23, N60, N61, N88, N90, N100, N108, N112, N113, N127, N128	resistant to all antibioticsbut sensitive to tobramycin, tetracycline, imipenem, meropenem, amikacin, ciprofloxacin, ceftazidime, cefalothin and pipracillin

As a conclusion, the higher isolation rate of *A. baumannii* was from ICU, more frequently from sputum specimens. The infection with *A. baumannii* was higher among males than females, and the more infected age group was 31-40 years. The higher isolation percentage of imipenem-resistant *A. baumannii* was from blood disease (leukemia) department, in particular from sputum specimens. All *A. baumannii* clinical isolates showed multidrug resistance; nevertheless, tobramycin and tetracycline recorded moderate resistance. The distribution of imipenem and other antibiotics resistance among environmental isolates were less than among *A. baumannii* clinical isolates. The imipenem resistant *A. baumannii* increased from 0% in 2006-2007 to 40.9% in 2010, and to 65.22% (resistant and intermediate) in 2011-2012.

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