Serological relatedness among clinical and environmental Acinetobacter baumannii isolates isolated from hospitals in Baghdad

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Abstract

During 2011, 1900 clinical specimens (urine, wounds, burns, blood and sputum) and 240 hospital environment specimens were collected from four hospitals in Baghdad/Medical city including: Baghdad Teaching Hospital, The Martyr Gazi Al-Hariry Hospital, Welfare Teaching Hospital and The Burn Specialist Hospital. All specimens were cultured and 128 Acinetobacter baumannii were obtained from clinical and environmental specimens in a ratio of 6.05% (n=115) and 5.42% (n=13), respectively. These isolates were identified using microscopic examination, biochemical tests and Api 20 E system.The slide agglutination technique for rabbit immune sera and A. baumannii bacteria was used and our data analysis revealed a serological relatedness among the isolates. We found 22 different serotypes for 128 clinical and environmental A. baumannii isolates. The most common serotype was serotype **1** which included 34 isolates (26.56%) and there were 40 (31.25%) miscellaneous isolates which isolated from different specimens, departments and hospitals. Notably, they didn't arrange within any of the 22 serotypes. Upon such findings we may conclude, there is a wide variation in serological characteristics of the locally isolated A. baumannii.

Keyword: Acinetobacterbaumannii, Serological relatedness

القرابة المصلية بين العزلات البيئية والسريرية لبكتريا Acinetobacter baumanniiالمعزولة من مستشفيات في بغداد

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الخلاصة

خلال عام ٢٠١١ ، ١٩٠٠ عينة سريرية (ادرار، جروح ، حروق ، دم وقشع) و٢٤٠ عينة من بيئة المستشفيات تم جمعها من أربع مستشفيات في بغداد من دائرة مدينة الطب شملت م. بغداد التعليمي ، م. الشهيد غازي الحريري ، م.حماية الأطفال التعليمي ومستشفى الحروق التخصصي. تم زرع جميع العينات وبعد نموها شخصت العزلات باستخدام الفحص المجهري والاختبارات الكيموحيوية ونظام Api 20 E. تم الحصول على ١٢٨ عزلة تعود الى بكتريا A. baumanniiوبنسبة6.05% (n=115) من العينات السريرية و 5.42% (n=13) من العينات البيئية. تم أستخدام فحص التلازن بالشريحة لأختبار تلازن كل من المصول الممنعة للأرانب كل أرنب مع بكتريا A. baumannii وقد أظهرت النتائج وجود قرابة مصلية بين العزلات، فقد وجد ٢٢ نمطا مصليا للعزلات ال١٢٨ السريرية والبيئية وسميت من١ – ٢٢. وكان النمط المصلي ١ هو النمط الأكثر شيوعا إذ تضمن ٣٤ عزلة وبنسبة (26.56%)، أيضا وجد إن ٤٠ عزلة متفرقة و بنسبة (31.25%) من نماذج وردهات ومستشفيات مختلفة لم تنتظم في أي نمط مصلي من الأنماط ال٢٢ ومن هذا نستنتج إن هناك تنوع واسع في الصفات المصلية في عزلاتA. baumannii المحلية.

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Introduction

Acinetobacter baumannii is a Gram-negative, non-motile, obligate aerobic coccobacilli that is commonly found in soil, water, sewage, and in healthcare settings [1 and 2] , during the past few decades, has evolved from an organism of questionable pathogenicity to one of the most important pathogens causing hospital-acquired infections (nosocomial infections), particularly in intensive care units (ICUs) [3].

Clinically, it is the most important species of the genus Acinetobacter [4 and 5] . It becomes one of the most difficult pathogens to treat [6], and become one of the leading nosocomial pathogens worldwide [7]. The outbreak of A. baumannii associated with United States military operations in Iraq generated special interest in this organism [8]. Features contributing to A. baumannii pathogenicity are resistance to a broad range of antimicrobial agents and to environmental stresses, persistence in the hospital setting, and the tendency for epidemic spread [9].

A study reported that it is very useful to employ the serological typing as epidemiology tool in the evaluation of A. baumannii dissemination in hospital units [10]. There is no available serotyping kit for serotyping A. baumannii but there were many trials for serotyping A. baumannii such as; polyclonal rabbit immune sera against A. baumannii strains were used [10 and 11] .

To our knowledge, no study has been performed in Iraq for serotyping of A. baumannii. Therefore, this study was aimed to study the serologic among clinical and environmental A. baumannii isolates.

Materials and Methods

Specimens collection

Through the period extending from first January 2011 till December 2011, one thousand and nine hundreds specimens comprising; urine, wounds, burns, blood and sputum, were collected from patients in sterilized containers fromfour hospitals in Baghdad/ Medical city including: Baghdad Teaching Hospital, The Martyr Gazi Al-Hariry Hospital, Welfare Teaching Hospital and The Burn Specialist Hospital.

At the same period of patients' specimens collection**,** two hundred and forty hospital environment specimens were collected from (patients' beds, tables, sinks, floors, air samples and medical equipments).

Isolation and identification of Acinetobacter baumannii isolates

In the laboratory under aseptic conditions, the collected specimenswere streaked directly on blood agar and MacConkey agar, incubated for 24 hours at37ºC. 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 hours at 37ºC [12].

Identification of A. baumannii isolates identified according to morphological and cultural characteristics as well as conventional biochemical tests. Identification was confirmed by API 20E system.

Serotyping relatedness test

Preparation of Acenitobacter baumannii antigens

For agglutination tests, cells of 128 A. baumannii isolates were prepared as follows. As a check for viability and purity, each isolates was subcultured onto Trypticase soy agar and incubated at 37°C overnight. Subsequently, 10 colonies of each isolates were picked and transferred to 25 ml of Trypticase soy broth and incubated at 37°C overnight. The cultures were then boiled for 1 hour. The cell suspensions were centrifuged at 2.500 rpm at 20°C for 10 min. The supernatants were discarded, and the cells were washed (twice) with 10 ml of sterile 0.145 M NaCl solution (this solution was prepared by dissolving 4.24 g of NaCl in 500 ml of D.W. to obtain 0.145 M NaCl. Thereafter it was autoclaved and stored at 4ºC). Afterward, the pellets were suspended in 5 ml of 0.145 M NaCl solution (bacterial cells number equivalent to 1.5×10^8 CFU/ml) and then stored at 4°C until further use [11].

Production of rabbit immune sera

Male and female New Zealand White rabbits (body weight, 1-2 kg) were immunized as described by Traub [11]. The rabbits (128 rabbits) received five intravenous injections (0.1, 0.2, 0.4, 0.8 and 0.8 ml) of A. baumannii antigens in which bacterial cells number equivalent to 1.5×10^8 CFU/ml (each rabbit immunized with one of the 128 A. baumannii antigens (the antigens were prepared for all 128 A. baumannii isolates)) which prepared and mentioned above into the marginal ear vein at 5 days intervals. At 5-7 days following the last intravenous injection, the animals were anesthetized with sodium hexobarbiturate and bled by cardiac puncture. The sera were separated and stored at -65°C.

Slide agglutination test

Acinetobacter baumannii isolates were cultured on to MacConkey agar at 37°C for 18-24 hours. Slide agglutination technique was used [13], which summarized as follows: portions of culture under test were emulsified into two separate drops of PBS on a glass slide, until a smooth and fairly dense suspension was obtained. To one drop of suspension, a drop of phosphate buffer saline (PBS) was added, mixed well and considered as a control. To the other suspension drop, a drop of undiluted rabbit immune sera was added and mixed by an aid of a stick.The slide was rotated for one minute. Thereafter, agglutination was observed by naked eye and recorded for each A. baumannii isolate.

Statistical analysis

Cluster analysis of serotype patterns was performed by the unweighted pair group method with arithmetic averages (UPGMA). Data analysis was performed with by using SPSS (Statistical Package for Social Sciences) 2008, version 17.

Results and Discussion

Isolation and identification of Acinetobacter baumannii isolates

Out of 1900 clinical specimens, 115 (6.05%) were identified as A. baumannii which was isolated in high percentage; ٣٨.٢٦% (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).

The environmental isolates of A. baumannii were diagnosed side by side with clinical isolates and out of 240 hospital environmental samples, 13 (5.42%) were belonged to A. baumannii. A fair similar result was recorded by a local study which found that out of 424 clinical specimens (urine, wounds, burns, blood and sputum), 34 (8.01%) were identified as A. baumannii, a result is fair closely to our result [14].

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.API system confirmed the results of morphological and biochemical tests.

Table 1-: Biochemical test results for Acinetobacter baumannii.

Id	Biochemical test	Result
١	Catalase production	+
٢	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

Serotyping relatedness test

The traditional slide agglutination technique for rabbit immune sera and A. baumannii bacteria was used in the test of serotyping relatedness for clinical and environmental isolates of A. baumannii, figure 1.

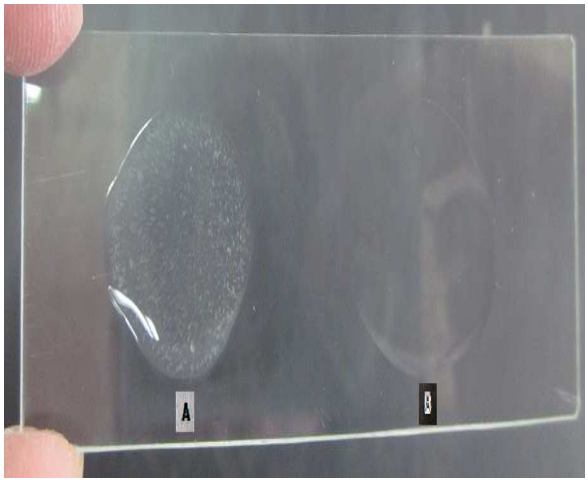

Figure 1- Slide agglutination test of rabbit immune sera and Acinetobacter baumannii bacteria, positive result (A) and negative result (B).

The data analysis showed a serological relatedness among the isolates; being their similarity (cut off point) was greater than 70%. Consequently, there were 22 different serotypes for 128 clinical and environmental A. baumannii isolates, as it illustrated in figure -2; named serotype 1 to 22.
The most common serotype was:

serotype1 which included 34 isolates (26.56%). Interestingly, most isolates belonged to serotype 1 were from sputum 19 (14.84%) isolates and 12 (9.38%) isolates from blood specimens. In addition to 3 (2.34%) isolates were collected from hospital environment.

Serotype 2 consisted of 3 (2.34%) isolates all them from blood specimens and from Baghdad teaching hospital.

Serotype3 contained 2 (1.56%) isolates of urine origin and all of them were collected from Baghdad teaching hospital.

Serotype 4 took account of 2 (1.56%) isolates, one of them from blood specimens; whereas the other one was taken from hospital environment.

Serotype 5 embraced 3 (2.34%) sputum isolates. All of them were isolated from Baghdad teaching hospital; particularly from Intensive care unit (ICU) department.

Serotype 6 consisted of 2 (1.56%) isolates from wound specimens. Both of them are from the same hospital (Baghdad Teaching Hospital); in particular from leukemia department.

Serotype 7 included 3 (2.34%) isolates all of which from sputum specimens, from ICU department in Baghdad teaching hospital.

Serotype 8 included 2 (1.56%) wound isolates; which was collected from surgical department in Welfare teaching hospital.

Serotype 9 included 3 (2.34%) isolates, originated from wound specimens, from surgical department in Baghdad teaching hospital.

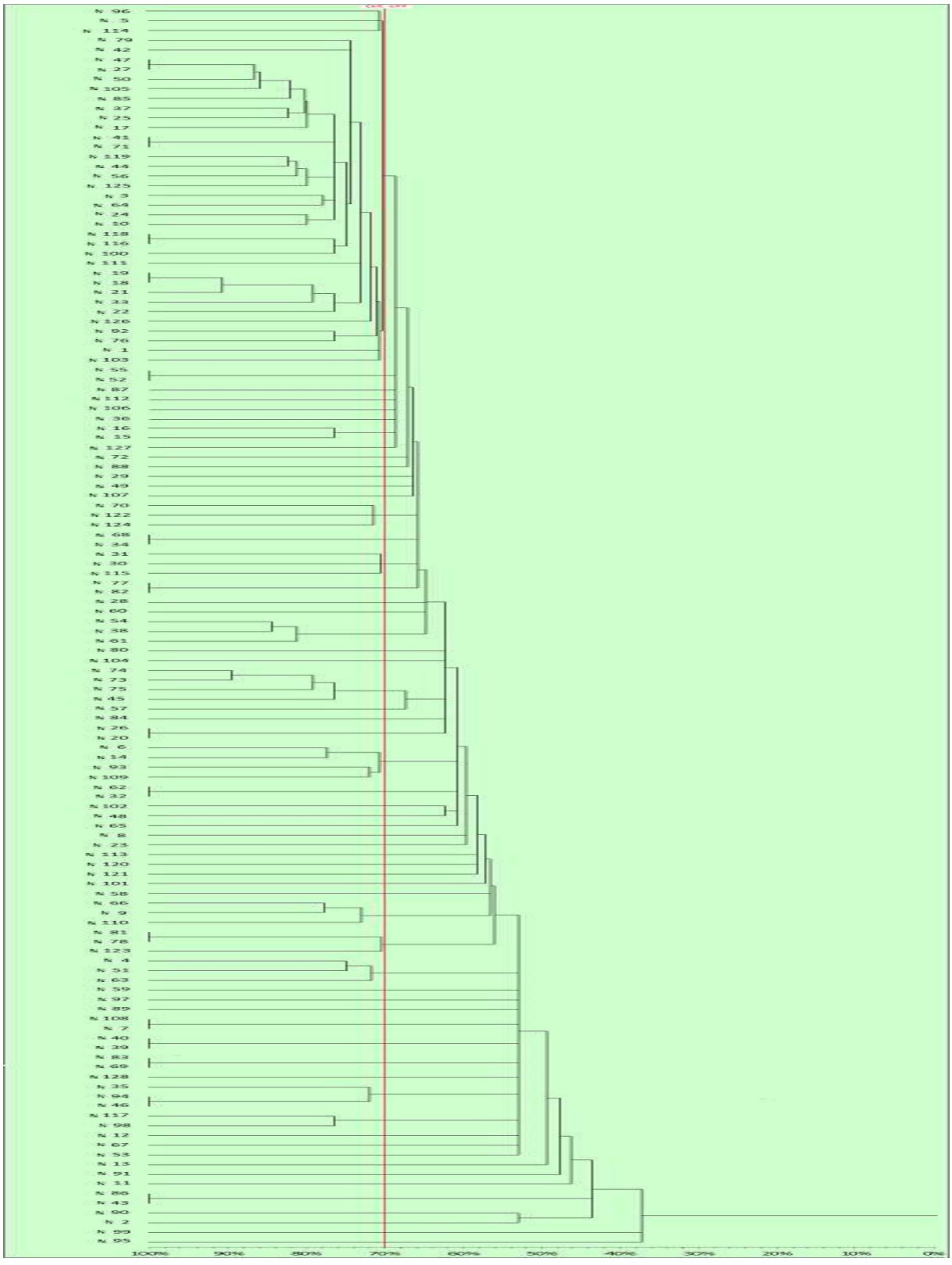

Figure 2- Dendrogram (cluster analysis) of 128 clinical and environmental Acinetobacter baumannii isolates serotyping patterns, clustering is based upon the Unweighted Pair Group Method with Arithmetic Averages (UPGMA), on a >70% similarity cut off point.

Serotype 10 included 4 (3.13%) isolates were isolated from burns specimens; all isolates were from the Burn Specialist hospital.

Serotype 11 included 2 (1.56%) isolates were isolated from blood specimens, from leukemia department in Baghdad teaching hospital.

Serotype 12 included 4 (3.13%) isolates. Three of them isolated from blood specimens of respiratory care unit (RCU) department and 1 isolate of them from hospital environment, 2 isolates from Baghdad teaching hospital and the other 2 were from Welfare teaching hospital.

Serotype 13 included 2 (1.56%) isolates of wound origin. Both were isolated form ICU department in Baghdad teaching hospital**.**

Serotype 14 contained 3 (2.34%) isolates. Two of them from blood specimens (ICU department) in addition to another isolate obtained from hospital environment. Moreover, 2 isolates from the Martyr Gazi Al-Hariry hospitalwhile the last isolate was from Baghdad teaching hospital.

Serotype 15 included 3 (2.34%) isolates. Two isolates were isolated from wound specimens in Welfare teaching hospital. The third one was isolated from sputum specimens in Baghdad teaching hospital.

Serotype 16 contained 3 (2.34%) isolates from sputum samples in Baghdad teaching hospital **(**RCU department).

Serotype 17 included 2 (1.56%) isolates obtained from Baghdad teaching hospital environment (environmental isolates).

Serotype 18 included 2 (1.56%) isolates which isolated from sputum specimens in Baghdad teaching hospital (RCU department).

Serotype 19 included 2 (1.56%) isolates of wound origin in ICU department in Baghdad teaching hospital.

Serotype 20 included 3 (2.34%) isolates obtained from RCU department in Baghdad teaching hospital.

Serotype 21 included 2 (1.56%) isolates originated from wound specimens which collected from in Baghdad teaching hospital.

Serotype 22 included 2 (1.56%) isolates of sputum origin. Both of them were isolated leukemia department in Baghdad teaching hospital.

However, there were 40 (31.25%) miscellaneous isolates which isolated from different specimens, departments and hospitals. Notably, they didn't arrange within any of previously mentioned 22 serotypes.

Upon such findings we may conclude that there is a wide variation in serological characteristics of the locally isolated A. baumannii, which might reflect the widely distribution and transmission of these isolates.

A

In conclusion, Serotyping relatedness of A. baumannii by the traditional slide agglutination procedure shows a wide variation in serological characteristics of the locally isolated A. baumannii, there were 22 different serotypes and 40 miscellaneous isolates among 128 clinical and environmental isolates.

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