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 I 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: Acinetobacter baumannii, Serological relatedness

القرابة المصلية بين العزلات البئنية والسبيبية لبكتيريا Acinetobacter baumannii المستشفيات في بغداد

الخليفة
خلال عام 2011، 1900 عينة سريرية (إدرار، جروح، حروق، دم وقثع) و240 عينة من بيئة المستشفيات تم جمعها من أربع مستشفيات في بغداد من دائرة مدينة الطب شملت: بغداد التعليمي، ومستشفى المريض الحريري، ومستشفى الأطفال التعليمي ومستشفى المريض التخصصي. تم تفريق جميع العزلات باستخدام فحص المجمهر والفحوصات الكيميائية والنظام E Api. تم التحقق على 128 عزلة تعود إلى بكتيريا A. baumannii. وحصيلة 6.05% (n=115) من العينات السريرية و5.42% (n=13) من العينات البئنية. تم استخدام فحص الثلاثة بالشريحة لأختبار تلاقى كل من العزلات و34 عزلة من A. baumannii المهمة للأمراض كل أربعة مع بكتيريا A. baumannii. وقد أظهرت النتائج وجود قرابة مصلية بين العزلات. وقد وجد 32 تعابير مضمونًا لعزلات السريرية البئنية وسميت من 1 إلى 22. وكان النطاق الأكثر شيوعًا 32 عزلة ونسبة (26.56%). أيضاً وجد 40 عزلة متفقة ونسبة (31.25%) من نتائج فحوصات ومستشفيات مختلفة لم تتسرع في أي نمط مصلي من الأحماض إلى 40 ونسبة هذا A. baumannii.

نستطيع إن هناك تنوع واسع في الصفات المصلية في عزلات A. baumannii.

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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 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.

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 equipment).
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; \( \gamma^\text{A} \), \( \gamma^\text{A} \) (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, No gas, No \( \text{H}_2\text{S} \). 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>
<thead>
<tr>
<th>Id</th>
<th>Biochemical test</th>
<th>Result</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Catalase production</td>
<td>+</td>
</tr>
<tr>
<td>2</td>
<td>Citrate utilization</td>
<td>+</td>
</tr>
<tr>
<td>3</td>
<td>Growth at 44°C</td>
<td>+</td>
</tr>
<tr>
<td>4</td>
<td>Hemolysin production</td>
<td>- (( \gamma ) hemolysis)</td>
</tr>
<tr>
<td>5</td>
<td>Indole production</td>
<td>-</td>
</tr>
<tr>
<td>6</td>
<td>Lactose fermentation</td>
<td>-</td>
</tr>
<tr>
<td>7</td>
<td>Motility</td>
<td>-</td>
</tr>
<tr>
<td>8</td>
<td>Oxidase production</td>
<td>-</td>
</tr>
<tr>
<td>9</td>
<td>Kligler iron agar (KIA)</td>
<td>Alkaline slant / No change bottom, No gas, No ( \text{H}_2\text{S} )</td>
</tr>
<tr>
<td>10</td>
<td>Urease production</td>
<td>-</td>
</tr>
</tbody>
</table>

+: 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.
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:

**Serotype 1** 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.

**Serotype 3** 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 hospital while 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.

References


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