Distribution of quorum sensing genes and typing of clinical Acinetobacter baumannii isolates by ERIC-PCR

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Abstract

Background: Acinetobacter baumannii has arisen as disturbing nosocomial pathogens between Iraqi hospitals inpatients. The aim of current study was to determine if the increase of A. baumannii incidence in patients on blaOXA-51 gene in A. baumannii that carry QS gene showed pathogenicity of clinical isolates and to determine the efficiency of ERIC-PCR fingerprinting method for genotyping of A. baumannii. Methods: Sixteen isolates diagnosed as A. baumannii and genetically confirmed by blaOXA-51 as a marker gene from different clinical sources in Baghdad hospitals. The virulence of the A. baumannii does not require, carrying full set of QS (LasR/RhlR, LasI/RhlI) genes. Results: The positive QS genes results were distributed from high to low expression, lasI 75%(45/60), 70%(42/60)for RhlR, 50%(30/60) for rhlI, and 13.3%(8/60) for LasR. Using fingerprinting ERIC-PCR analysis, 57 isolates of A. baumannii were clustered into 2 groups while the remaining 3 were single isolates. The genetic linking of A. baumannii isolated from different hospitals inpatients was high, indicating horizontal gene transfers within hospitalized patients. Conclusion: Our findings indicated accurate and fast diagnosis method to detect virulent A. baumannii isolates harboring differing sets of QS by using blaOXA-51 gene and ERIC-PCR for genetic variations, respectively, possible to be helpful with epidemic infections. Keywords: Acinetobacter baumannii, Quorum sensing, blaOXA-51, ERIC-PCR

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Introduction

Acinetobacter baumannii is an important Gram-negative coccobacilli classified as an opportunistic bacterial pathogen that is the cause of nearly about 9% of all nosocomial infections in most hospitals[1]. A large number of severe nosocomial infections are thought to be caused by A. baumannii involving a variety of systemic infections in patients occupying intensive care units with high mortality rates[2]. Various virulence factors of A. baumannii have made it difficult to treat or control its spread. Many reports indicated that a system set gene called Quorum Sensing (QS) possess a critical function in aggressiveness of these bacteria[3]. The identification of clinical A. baumannii generally completed using both cultural experiments and chemical analysis, and overnight at 37°C and 44°C growth behavior[4]. In order to get best understanding of clinical significance and epidemiology.
of *Acinetobacter* species, different molecular methods have been improved, yet mostly complicated for approval of diagnostic implementation in the microbiological laboratories\[5\]. Numbers of methods for typing were developed to reach this aim, according to phenotypic tests and molecular techniques for different typing\[6\]. Molecular typing systems became certified like ribotyping, pulsed-field gel electrophoresis plasmid profile and enterobacterial repetitive intragenic consensus (ERIC)\[7\]. A wider rise in incidence of *A. baumannii* outbreaks has been observed at Baghdad Hospitals, Iraq.

The aim of current study was to determine if the increase of *A. baumannii* incidence in patients on *bla*OXA-51 gene in *A. baumannii* that carry QS gene showed pathogenicity of clinical isolates and to determine the efficiency of ERIC-PCR fingerprinting method for genotyping of *A. baumannii*.

**Material and Methods**

**Bacterial isolation**

Sixty isolates of *Acinetobacter baumannii* were identified from a variety of patients' specimens, including 25 from burn sites, 29 from urinary tract infections and 6 from blood samples, from many hospitals in Baghdad. Bacteria were obtained from patients' samples, and from the outpatients' department samples collection continued from July 2017 till December 2018. The isolates were identified as *Acinetobacter baumannii* depending on biochemical method in the Api 20NE system (bio Merieux).

**DNA Extraction**

In order to check purity on nutrient agar plates, all isolates were grown on nutrient broth for 24h at 37°C. About two loops worth of biomass from agar plates were scratched, then suspended in 150 microliter of deionized water, after that boiled for 10min. About 5 microliter (as template for PCR) was transferred to PCR tube after 10 minute under 10000× g centrifugation at 4°C\[8\].

**Quorum sensing systems**

The isolates were identified as *Acinetobacter baumannii* depending on biochemical method in the Api 20NE system (bio Merieux) \[9\].

**PCR Application**

For the purpose of confirming the isolates as *Acinetobacter baumannii*, PCR technique was used which is constructive on housekeeping gene (*OXA51* gene) sequence with specific designed primers by using Primer 3 programs (Accession:LC093481.1). Those used in this study were F: 5’-AGATTATAAGTGGAAGGGCA-3’ and R: 5’-CACTCATCTTGGGACTTCCTTG-3’. PCR was accomplished in 25μL reaction volume consisted of template DNA 5μl, 12.5μl of GoTaq® Green Master Mix, forward & reverse primers (1.5μl for each), finally 4.5 microliter of Nuclease-Free water was then added to the mixture to reach final volume of 25μl. PCR was operated
under the following conditions: primary denaturation step at 95°C for 5min, 32 repeated cycles with denaturation starting step at 94°C for 30sec, annealing at 58°C for 40sec, and finally 1min at 72°C as extension step, final extension step at 72°C for 7min.

**DNA amplification using ERIC-finger printing PCR**

Forward primer (5’-ATGTAAGCTCCTGGGGATTCAC-3’) and reverse primer (5’AAGTAAGTGACTGGGGTGAGCG-3’) had been used for amplifying repetitive sequences found in the chromosomal DNA of *Acinetobacter baumannii* isolates according to [10].

**Dendrogram analysis and genetic linkage**

The dendrogram was constructed depending on the banding pattern produced by ERIC-PCR. A bilateral table or a haplotype matrix for every strain was constructed by longitudinal structuring presence (1)/absence (0) data came from analysis of the gel/antibiogram was submitted for statistical analysis by Dice coefficient values [11].

**Statistics**

The level of genetic similarity among the strains was cleared by constructing dendrogram expressing.

**Results and Discussion**

Phonotypical and biochemical confirmation were done for sixteen isolates and diagnosed as *A. baumannii* which were obtained from different clinical sources in addition to hospitals in Baghdad. All 60(100%) isolates showed the gene of interest (Figure 1).

Figure (1): Agarose gel electrophoresis (1% agarose, 7V/cm^2^ for 60min) of *blaOXA-51* (300bp amplicon). Lane M: 100bp DNA ladder. Lanes (1-12) represent the gene bands.

**Distribution of Quorum Sensing Genes**

Sixty isolates from different sources and hospitals in Baghdad used to study quorum sensing genes (*lasI, lasR, rhII, RhIR*) which were screened by multiplex PCR technique and the results showed that 90% (54/60) were positive for one or more quorum sensing genes while only 10% (6/60) were negative.
for all these genes. The positive QS genes results distributed as follows: 70% (42/60) for RhIR, 50% (30/60) for rhII while 13.3% (8/60) for LasR and the lasI 75% (45/60). This result indicated the pathogenicity of Acinetobacter baumannii, in the manner role of the QS as controlled genes for a variety of virulence factors\cite{12}. These result were in respective agreement with Abed and Ali,\cite{13} who confirmed Quorum sensing genes in different pattern of Acinetobacter baumannii isolates and indicated their role in aggressiveness of bacteria. The different distribution of QS genes may belong to point mutation that block the expression or may be expressed in low level. Moreover,\cite{14} explained these results and confirmed that point mutation in quorum sensing gene caused that result and reported QS mutation isolates that were incapable of producing the signal molecule in the quorum sensing genes but remain make infections in humans. On the other hand,\cite{15} explained that there may be another virulence factors which may not be necessarily controlled by QS. The results of this study noticed that the QS genes have an essential role in the pathogenesis of Acinetobacter baumannii and indicated that despite of quorum sensing deficient, Acinetobacter baumannii were able to cause clinical infections in humans and this contradicted the theory that QS perform a critical role in Acinetobacter baumannii pathogenicity and not all virulence factors controlled by quorum sensing.

ERIC–PCR typing
ERIC-PCR had fingerprinting grouped A. baumannii strains isolated from different specimens and from the same period of isolation and location. In PCR, ERIC primer sequence was used for detecting the differences in number and distribution of that bacterial repetitive sequence in clinical isolates of A. baumannii genomes. ERIC sequences detection by PCR produced nine DNA segments ranged from 300bp to 2900bp with variable segments (Table 2). The dendrogram of similarity obtained by ERIC-PCR showed the presence of two main clusters (A-B); cluster B predominated and contained 33 isolates with (1-3) same bands making 4 real clones with 8 unique patterns. On the other hand, cluster A contains 24 isolates with (2-5) same bands forming 3 real clones, of these, 14 clones generated and 8 unique patterns while the remaining 3 isolates identified in a unique cluster. We also verified the fact that same patient can have gnomically special isolates located in either the same cluster or in different one. The present results confirmed that the isolates collected from same hospitals could be related rather than unrelated so that transmission of A. baumannii from patient-to-patient can cause propagation of clonal strains to another wards of the hospital. So, nosocomial transmission can be avoided with simple hygienic controls, avoiding contaminated materials, taking care of cleanness and segregation of patients.

ERIC-PCR technique performs useful technique for the epidemiological typing of nosocomial bacteria due to their simplicity and speed compared with other molecular technique. This result was similar to that mentioned by Kidd et al. (2011) that ERIC–PCR technique can catch a chromosome-wide view on diversity, with differences between strains according to genetic changes, however, the specific causes for any variation can only be supposed.
Table (1): Molecular weight and percentage values of the bands obtained in ERIC-PCR

<table>
<thead>
<tr>
<th>Band No.</th>
<th>Molecular weight</th>
<th>Percentage value</th>
</tr>
</thead>
<tbody>
<tr>
<td>ERIC1</td>
<td>300</td>
<td>80%</td>
</tr>
<tr>
<td>ERIC2</td>
<td>400</td>
<td>72.72%</td>
</tr>
<tr>
<td>ERIC3</td>
<td>500</td>
<td>58.18%</td>
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<tr>
<td>ERIC4</td>
<td>700</td>
<td>40%</td>
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<td>ERIC5</td>
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<td>1900</td>
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<td>2000</td>
<td>14.54%</td>
</tr>
<tr>
<td>ERIC9</td>
<td>2100</td>
<td>18.18%</td>
</tr>
<tr>
<td>ERIC10</td>
<td>2900</td>
<td>40%</td>
</tr>
</tbody>
</table>

Conclusion

In the present study, ERIC – PCR established fingerprinting techniques were used to analysis the diversity of *A. baumannii* isolates in clinical specimens depending on genetic manner. The isolates were genetically variable as 16 major ERIC - PCR clonal groups and 7 unique genotypes were obtained (Figure 2). The high heterogeneous strains were isolated from UTI.

Figure (2): Agarose gel electrophoresis (1% agarose, 7V/cm² for 60min) of ERIC-PCR. Lane M: 100bp DNA ladder. Lanes 1-12 represent gene bands.
Figure (3) Dendrogram of ERIC using BioNumerics fingerprint data software and not weighed pair groups, Dice method in addition to arithmetic averages at 97% similarity on 60 of Acinetobacter baumannii.

Ethical Clearance: The research Ethical Committee at scientific research by ethical approval of both environmental and health and higher education and scientific research ministries in Iraq.

Conflict of interest: The authors declare that they have no conflict of interest.

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