Isolation, molecular identification and antimicrobial susceptibility of Acinetobacter baumannii isolated from Baghdad hospitals

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Abstract- Increasing resistance to antimicrobial agents used in the treatment of burns and wounds infections with Acinetobacter baumannii strains has become an important concern. Isolation and identification of A. baumannii strains from patients with burns and wounds infections by conventional and molecular methods and detection of their antimicrobial susceptibility. Antibiotics resistance of 96 A. baumannii strains isolated from the samples collected between February 2015 and June 2015 were evaluated. Identification of A. baumannii isolates were determined by CHROMagar Acinetobacter, API 20E and automated system VITEK 2 and molecular methods by detection 16S rRNA and blaOXA-51 genes. Antimicrobial susceptibility tests were performed by agar disk diffusion and broth microdilution methods. Among 476 samples included burns and wounds infections, 96 isolates of A. baumannii were obtained, 65 belonged to burns and 31 from wounds. CHROMagar Acinetobacter had 100% sensitivity and specificity for isolation of A. baumannii. The PCR results showed that 16S rRNA sequence and blaOXA-51 gene exists in all A. baumannii collected from positive samples, and this confirmed the accuracy of other identification tests. In the present study, 87.5% of the isolates had Multi-Drug Resistance (MDR). It has been found that A. baumannii strains had high resistance to most antibiotics used: Cefotixime (87.5%), Ciprofloxacin (80.2%), Amikacin (79.2 %), Meropenem (75%) Imipenem (81.3%) and Pipracillin (81.3%) but low resistance to Tigecycline (11.5%) and Colistin (7.3%). The present results indicate that antimicrobial resistance of A. baumannii in Iraq has increased, which may very well affect the antimicrobial resistance of this organism worldwide. Therefore their early detection is essential for stimulates effective treatment in intensive care units. Detection of blaOXA-51-like gene and CHROMagar Acinetobacter can be used as a simple and reliable method to differentiate A. baumannii strains.

Index Terms- Acinetobacter baumannii, Antimicrobial susceptibility, molecular identification, burns, wounds.

I. INTRODUCTION

Acinetobacter baumannii is one of the most important opportunistic pathogens that cause outbreaks in hospitals and serious health care associated complications in hospitalized patients [1]. A. baumannii has become endemic in hospitals due to its versatile genetic machinery, which allows it to quickly evolve resistance factors, and to its remarkable ability to tolerate harsh environments [2]. During the past decade, nosocomial outbreaks of A. baumannii have been described with increasing frequency, occurring mostly in intensive care, burn and surgical units [3,4]. A. baumannii infections mainly affect patients with severe underlying disease, and are associated with major surgery, burns or trauma. Most studies report high overall mortality rates in patients with A. baumannii bacteremia and may be associated with considerable morbidity and (overall) mortality as high as 58% [5,6]. The increasing prevalence of multi-drug resistant in A. baumannii strains, have caused A. baumannii become the most important pathogen after Pseudomonas aeruginosa among non-fermentative gram-negative bacteria [7].

The most alarming problems encountered are the ability of this species to have different mechanisms of resistance and the emergence of strains that are resistant to all commercially available antibiotics coupled with the lack of new antimicrobial agents, this has resulted in a limited choice of antibiotics for treatment of multidrug resistant isolates of A. baumannii [8,9]. There were increasing number of hospital outbreaks caused by A. baumannii has been reported from several countries around the world. In addition, inter-hospital spread of multidrug resistant A. baumannii has been observed as well as spread among countries [10]. A number of resistance mechanisms to many classes of antibiotics are known to exist in A. baumannii, including β-lactamases, multidrug efflux pumps, aminoglycoside modifying enzymes, permeability defects, and the alteration of target sites [11].

This study was designed to isolation and identification of A. baumannii from burns and wounds infections of hospitalized patients in Baghdad hospitals using conventional and molecular methods, also this work aims to investigate antimicrobial susceptibility patterns among different A. baumannii isolates.

II. MATERIALS AND METHODS

Bacterial isolates

A total of ninety six A. baumannii isolates were collected from 476 clinical specimens such as burns and wounds from patients at Baghdad hospitals, Iraq during the February –July 2015 period. Bacterial isolation and identification were performed using standard laboratory methods. The isolates were
non-repetitive, meaning that each isolate was obtained from a particular patient and each patient was sampled only once. Samples were streaked across CHROMagar Acinetobacter (CHROMagar, France), MacConkey and blood agar plates for all specimens. Presumptive identification was done based on culture characteristics, gram stain and conventional biochemical tests [12].

Standard identification, confirmation and complete method were conducted including using API 20E (bioMérieux, France) and Vitek 2 system (bioMérieux, France) with ID-GNB card for identification of gram-negative bacilli, according to the manufacturer’s instructions. Samples confirmed as A. baumannii were stored in Tryptic Soy Broth (Merck, Germany) containing 20 % glycerol at -20 °C and were subjected to further molecular identification. The PCR of blaOXA-51-like genes was used as a final confirmation as to the presence of A. baumannii species [13].

Antimicrobial susceptibility testing

Antimicrobial susceptibility tests were performed by agar disk diffusion, according to manufacturer instructions and Clinical and Laboratory Standards Institute (CLSI) guidelines [14]. The applied antimicrobials were as follows: Amikacin (30μg), Gentamicin (10μg), Imipenem (10μg), Meropenem (10μg), Ceftazidime (10μg), Cefotaxime (30μg), Ciprofloxacin (5μg), Levofloxacin (5μg), Tetracycline (10μg), Tigecyclin (30μg), Aztreonam (30μg), Ticarcillin - clavulanic acid (75/10μg), Oxacillin (1μg) Piperacillin (30μg), Trimethoprim / Sulfamethoxazole (25μg) and Colistin (10μg) (MAST Diagnostic Co., UK). For Tigecycline, the Food and Drug Administration (FDA) breakpoints for Enterobacteriaceae (14mm, resistant; 19mm, susceptible) were used. The growth inhibition zones around each disk were measured. For minimal inhibitory concentration (MIC) determinations, broth microdilution method Mueller-Hinton broth was used according to the CLSI guidelines [15]. Pseudomonas aeruginosa ATCC- 27853 were used as quality reference strains.

MDR A. baumannii was defined as an intermediately-resistant or resistant isolate to more than two of the antimicrobial agents

DNA extraction

Genomic DNA was extracted by standard DNA Extraction Kit (Promega, USA) according to the manufacturer’s instructions. The purity of DNA was evaluated by calculating the ratio of the absorbance at 260 and 280 nm (A260/A280), DNA concentration and 260/280 ratios are determined using a NanoDrop ND-1000.

Detection of 16s ribosomal RNA gene

Polymerase chain reaction (PCR) amplification of the 16S ribosomal RNA was performed in a DNA thermal cycler, (Applied Biosystem, Singapore) with the following cycling program: Initial denaturation at 95°C for 3 min, and 30 cycles of denaturation at 95°C for 1 min, annealing at 55°C for 1 min, extension at 72°C for 1 min and a final extension at 72°C for 5 min using primer sequence: [16]

Forward, 5- CAGCTCGTGTGAGATGTG-3
Reverse, 5- CGTAAGGCGCATGACTT-3

Detection of bla OXA 51 carbapenemase gene

bla OXA-51 gene was amplified as described (Woodford et al., 2006) [17]. Amplified DNA fragments were purified with Qiaquick PCR purification kits (Qiagen, USA). The PCR analysis was performed using the primer sequence:

OXA-51-like forward 5 -TAA TGC TTG GAT CGG CTT TG-3
OXA-51-like reverse 5 -TGG ATT GCA CTT CAT CTT GG -3

The amplification conditions were: initial denaturation at 94°C for 5 minutes, 30 cycles of 94°C for 45 seconds, 52°C for 40 seconds, 72°C for 45 seconds, and a final elongation at 72°C for 6 minutes

Amplified fragments were separated by electrophoresis in 1% agarose gel at 70V for 2hr. Finally, fragments were stained with ethidium bromide and detected under UV light.

III. RESULTS

Among 476 samples included burns and wounds infections, 103 samples were identified as Acinetobacter sp. 96 isolates (20.2 %) identified as A. baumannii. 65 samples were from burns and 31 samples were from wounds. The results of distribution of A. baumannii isolates in clinical samples based on type sample are shown in Table 1.

Table 1. Distribution of A. baumannii and MDR A. baumannii isolates in clinical samples (burns and wounds).

<table>
<thead>
<tr>
<th>Sample type</th>
<th>No. of (%) Sample</th>
<th>No. of A. baumannii (%)</th>
<th>No. of MDR A. baumannii (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Burns</td>
<td>315 (66.2 %)</td>
<td>65 (20.6 %)</td>
<td>57 (18.1 %)</td>
</tr>
<tr>
<td>wounds</td>
<td>161 (33.8 %)</td>
<td>31 (19.3 %)</td>
<td>27 (16.8 %)</td>
</tr>
<tr>
<td>Total</td>
<td>476</td>
<td>96 (20.2 %)</td>
<td>84 (17.6 %)</td>
</tr>
</tbody>
</table>

Of the 96 isolates of A. baumannii from the clinical samples, 65 belonged to burns (20.6 %). The wounds also had a high relative proportion of A. baumannii isolates (19.3 %). CHROMagar Acinetobacter was used for specific isolation of A.
*A. baumannii.* On CHROMagar isolates of *A. baumannii* appeared as bright red colonies at 24 hour as shown in figure 1, this medium also has selectivity for MDR *A. baumannii* by adding MDR Acinetobacter screening supplement. The results of conventional biochemical tests and MacConkey agar compared to CHROMagar Acinetobacter. CHROMagar Acinetobacter recovered all 96 isolates (100%). It had 100% sensitivity and specificity for isolation of *A. baumannii*. This medium also has selectivity for multidrug resistant *A. baumannii* (MDRAB).

The PCR results showed that 16S rRNA sequence exists in all 103 *Acinetobacter* sp. collected positive samples, and this confirmed the accuracy of biochemical tests. The results of PCR of blaOXA-51 gene showed that this gene exists in all 96 isolates which had been diagnosed as *A. baumannii*. These results may reflect more accurate and more sensitive detection of molecular diagnosis in comparison with CHROMagar Acinetobacter and biochemical tests. Figure 2 shows the results of PCR of the 16S rRNA gene. Figure 3 shows the PCR results of the bla OXA-51 gene.

In the present study, 87.5% of the isolates had multidrug resistance. It has been found that *A. baumannii* strains had high resistance to Cefotxime (87.5%), Imipenem (81.3%), Pipracillin (81.3%), Ciprofloxacin (80.2%), Amikacin (79.2%) and Meropenem (75%), but low resistance to Tigecycline (11.5%) and Colistin (7.3%).

**Figure 1.** Colonies of *Acinetobacter baumannii* on CHROMagar Acinetobacter.

**Figure 2.** Ethidium bromide stained agarose gel (2%) of PCR amplified products from extracted DNA of *A. baumannii* isolates and amplified with primers of 16S rRNA gene. Lane (M): DNA molecular size marker (100bp ladder), Lane (1-5): show a positive result with positive bands of 150bp 16S rRNA gene of *A. baumannii*, Lane (6): show a positive result for *A. lwofii*, lane (7): Negative control, (70V for 2hr).

**Figure 3.** Ethidium bromide stained agarose gel (2%) of PCR amplified products from extracted DNA of *A. baumannii* isolates and amplified with primers of bla OXA-51 gene. Lane (M): DNA molecular size marker (100bp ladder), lane (1): Negative control. Lane (2-9): show a positive result with positive bands of 353bp. (70V for 2hr).
Table 2. Percentages of antimicrobial susceptibility rate of 96 A. baumannii isolates against 16 antimicrobial agents.

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Resistant</th>
<th>Intermediate</th>
<th>Sensitive</th>
</tr>
</thead>
<tbody>
<tr>
<td>Amikacin AK</td>
<td>76 (79.2%)</td>
<td>2 (2.1%)</td>
<td>18 (18.8%)</td>
</tr>
<tr>
<td>Gentamicin GM</td>
<td>75 (78.1%)</td>
<td>11 (11.5%)</td>
<td>10 (10.4%)</td>
</tr>
<tr>
<td>Imipenem IPM</td>
<td>78 (81.3%)</td>
<td>8 (8.3%)</td>
<td>10 (10.4%)</td>
</tr>
<tr>
<td>Meropenem MEM</td>
<td>72 (75%)</td>
<td>11 (11.5%)</td>
<td>13 (13.5%)</td>
</tr>
<tr>
<td>Ceftazidime CAZ</td>
<td>60 (62.5%)</td>
<td>11 (11.5%)</td>
<td>25 (26%)</td>
</tr>
<tr>
<td>Cefotaxime CTX</td>
<td>84 (87.5%)</td>
<td>6 (6.3%)</td>
<td>6 (6.3%)</td>
</tr>
<tr>
<td>Ciprofloxacin CIP</td>
<td>77 (80.2%)</td>
<td>8 (8.3%)</td>
<td>11 (11.5%)</td>
</tr>
<tr>
<td>Levofloxacin LVX</td>
<td>72 (75%)</td>
<td>3 (3.1%)</td>
<td>21 (21.9%)</td>
</tr>
<tr>
<td>Tetracycline TE</td>
<td>50 (52.1%)</td>
<td>0 (0%)</td>
<td>46 (47.9%)</td>
</tr>
<tr>
<td>Tigecycline TGC</td>
<td>11 (11.5%)</td>
<td>7 (7.3%)</td>
<td>78 (81.3%)</td>
</tr>
<tr>
<td>Aztreonam ATM</td>
<td>74 (77.1%)</td>
<td>4 (4.2%)</td>
<td>18 (18.8%)</td>
</tr>
<tr>
<td>Ticarcillin–K clavulanic acid; TCC</td>
<td>67 (69.8%)</td>
<td>6 (6.3%)</td>
<td>23 (23.9%)</td>
</tr>
<tr>
<td>Oxacillin OX</td>
<td>80 (83.3%)</td>
<td>8 (8.3%)</td>
<td>8 (8.3%)</td>
</tr>
<tr>
<td>Piperacillin PI</td>
<td>78 (81.3%)</td>
<td>6 (6.3%)</td>
<td>12 (12.5%)</td>
</tr>
<tr>
<td>Trimethoprim– sulphamethoxazole TS</td>
<td>81 (84.4%)</td>
<td>10 (10.4%)</td>
<td>5(5.2%)</td>
</tr>
<tr>
<td>Colistin CO</td>
<td>7 (7.3%)</td>
<td>5 (5.2%)</td>
<td>84 (87.5%)</td>
</tr>
</tbody>
</table>

IV. DISCUSSION

The bacterium A. baumannii is a major hospital acquired pathogenic microorganism and usually affecting patients who are immunocompromised and those patients hospitalized in intensive care and burns units [18,19]. In this study, frequency of A. baumannii in burns was determined by 20.6 %. Hussein et al., [20] determined the frequency of A. baumannii by 5.22 % in burns specimens in intensive care units of Baghdad hospitals. In a study conducted by Mamani et al., [21] in Hamedan, frequency of Acinetobacter in burn wounds were about 16.6%. Previous study in Hilla hospitals including frequency of A. baumannii in wounds (3.12%) was lower than our results [22].

CHROMagar Acinetobacter is a recently developed selective agar for the rapid identification of MDRA. It contains agents which inhibit the growth of most gram-positive organisms as well as carbapenem-susceptible gram-negative bacilli; Gordon
et al., [23] results of CHROMagar Acinetobacter were compared with a molecular assay resulting in sensitivity and specificity of culture compared to PCR of 91.7% and 89.6%, respectively. 16S rRNA gene used for bacterial identification that is more robust, reproducible, and accurate than that obtained by phenotypic testing, hence Acinetobacter clinical isolates were identified by detecting the bacteria 16S rRNA genes [24,25].

blaOXA-51 genes are factors of resistance to carbapenem antibiotics in Acinetobacter which exists inherently in all A. baumannii strains and is chromosomal. The existence of the bla OXA-51 gene was investigated to prove A. baumannii strains [26,27]. The results indicated the presence of this gene in all the strains of A. baumannii and consistent with previous research [26-28]. Based on the results of antibiotic susceptibility (Table 2), among 96 samples of A. baumannii, 84 samples were resistant to more than 3 classes selected antibiotics, in other words, MDR A. baumannii (table 2).

The percentage of multidrug resistant isolates were in agreement with the results obtained by Ronat et al. [29] who found that multidrug-resistant organisms were common invasive pathogens in burn-injury patients in northern Iraq. Multiple factors have contributed to antimicrobial resistance in clinical A. baumannii isolates, since MDR A. baumannii strains have multitudes of resistance genes that A. baumannii may possess along with the potential horizontal gene transfer between polyclonal MDR A. baumannii strains. The presence of class 1 integrons and ISAba1 elements are always linked to the epidemic potential of A. baumannii [30,31].

Antibiotic resistance in A. baumannii is increasing at an alarming rate leading to increased morbidity, mortality and treatment costs in intensive care units settings as revealed by surveillance studies from over the last years [32].

V. CONCLUSIONS

These results revealed that A. baumannii incredibly improve resistance against antibiotics therefore the number of effective antibiotics will reduce dramatically and A. baumannii will cause serious health problems worldwide by time. The prevalence of A. baumannii infections causing the majority of burns and wounds infections is rising and the resistance against antibiotics is unpredictable globally.

REFERENCES


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