Comparison of three diagnostic methods for *Acinetobacter baumannii* Isolated from Baghdad Hospitals

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**ABSTRACT**

**Background and Objectives:** Precise identification of *Acinetobacter baumannii* in clinical diagnostic laboratories is difficult since the commonly used commercial identification systems based on phenotypic tests may not cover the diversity of the genus and because several species are difficult to differentiate phenotypically, rapidly identify infected with *A. baumannii* may improve the efficiency of infection control practices. The aim of this study was to compare three diagnostic methods for *A. baumannii* isolates to reduce the time and cost of diagnosis method and shorten the time of delivery of antibiotic therapy for patients and reduce risk.

**Materials and Methods:** From February 2016 to August 2016, 55 gram negative bacteria isolates were collected from three main hospitals in Baghdad. *A. baumannii* isolates were diagnosed by three different methods, including Chromagar Acinetobacter, and Chromagar Orientation, Vitek 2 system, and 16s RNA, RecA, gldA detection by PCR. **Results:** Out of 55 gram negative bacteria isolates, Chromagar Acinetobacter with Chromagar Orientation, Vitek 2, PCR identified *Acinetobacter* species (45 *A. baumannii* with 2 *Acinetobacter radioresistans*), 45 *A. baumannii* isolates respectively. The sensitivity and time for Chromogenic media, Vitek 2 and PCR, was 100% (24hr.), 81.8% (8hr.), 100% (3hr.) respectively. **Conclusion:** PCR robust method for diagnosis *A. baumannii* in clinical laboratory where a Vitek 2 system and Chromogenic media need more time and routine test.

**KEYWORDS:** *Acinetobacter baumannii*, Chromogenic media, Vitek 2 system, PCR.

**INTRODUCTION**

Most infections with *A. baumannii* involve organ systems that contain high levels of fluids. Such systems include among others the urinary and respiratory tract, peritoneal cavity, and are linked to indwelling devices. The difference between the infection and colonization with *A. baumannii* is difficult to differentiate. It is believed that the retrieval of *A. baumannii* in the hospitalized patient is a sign of severe illness, with a related mortality of about 30% [1].

Automated systems can identify this organism correctly to the species level, most participants reported using one of the following methods: Vitek1, Vitek2, MicroScan, BD Phoenix, API-20E, API-20NE, with and without classical tests [2].

Nevertheless, four species *A. calcoaceticus*, *A. baumannii*, *Acinetobacter* genomomic species 3 and *Acinetobacter* genomomic species 13TU – must be distinguished using molecular techniques [3].

Chromogenic media that can rapidly identify patients colonized or infected with *A. baumannii* may improve the efficiency of infection control practices, shorten the time of delivery of appropriate antibiotic therapy for infected patients and reduce mortality [4, 5]. *A. baumannii* clinical isolates are commonly resistant to multiple...
antimicrobial drug classes and have the ability to survive in the environment for prolonged periods of time, which facilitates their persistence in hospitals [6, 7], has become an increasingly prevalent cause of nosocomial infections especially immune-compromised and in Intensive Care Units (ICUs) patients in the last few years (8, 9). In this study, we aimed to compare three diagnostic methods for A. baumannii isolates to reduce time and cost of diagnosis method and shorten the time to delivery of antibiotic therapy for patients and reduce risk.

MATERIALS AND METHODS

**Isolation and diagnosis of A. baumannii:**

From February 2016 to August 2016, specimens were collected from infected patients with infections (UTIs), wounds, and sputum. Each swab taken carefully from the site of infection and placed in tubes containing ready-made media to maintain the swab wet during transferring to laboratory. Each specimen was immediately inoculated on the CHROMagar Acinetobacter™ was prepared without and with supplementation were dispersed slowly 32.8 g of powder base (B) in 1 Liter of purified water then added 4.0 ml of the liquid supplement AC092(S) into slurry and one more time with MDR supplement CR102 1 vial in 1 Liter according to the manufacturer’s instructions then inoculated media were incubated at 37°C in aerobic conditions and evaluated after 18-24 hr. Also CHROM orientation™ were dispersed slowly 33g of powder base in 1 Liter of purified water and Stir until agar is well thickened. These media were examined for presumptive colonies in accordance with the manufacturer’s recommendations.

**Vitek 2 system:**

All bacterial suspensions were taken from primary isolation plates were adjusted to a McFarland standard of 0.5 in 2.5 ml of a 0.45% sodium chloride solution with the VITEK 2 Densi-Chek™ instrument (bioMe´rieux). The time between preparation of the inoculum and the filling of the card was always less than 30 min. The format of the GN plastic card contains 47 tests. The GN is a fully closed system to which no reagents have to be added. The card was put on the cassette designed for VITEK 2, placed in the instrument, automatically filled in a vacuum chamber, sealed, incubated at 35.5°C, and automatically subjected to a colorimetric measurement by use of a new optical reading head every 15 min for a maximum incubation period of 10 hr. Data were analyzed using VITEK 2 database version 4.01, which allows for organism identification in the kinetic mode after 6-8 hr. of incubation, and using quality controlled standard strains (Acinetobacter baumannii ATCC BAA-747) obtained from American Type Culture Collection.

**Genotype diagnosis of A. baumannii by PCR:**

16s rRNA, gltA, and recA gene (a housekeeping gene) was used for genotypic diagnosis. Specific primers listed in table 1 were employed and the amplified size was 240 bp, 722 bp and 425 bp respectively. Template DNA was prepared by boiling method by Ruppé et al. [10]. Briefly, some isolated colonies of overnight growth bacteria were suspended thoroughly in 1 mL distilled water and boiled in a water bath for 6-10 min. After centrifugation, supernatant was used as template DNA. PCR mixture composed from 12.5 of GoTaq® Green Master Mix (2x) USA (Promega), 5 μl template DNA, 1.5 μl primers (for each) final concentration (0.6 pmol/μl), and nuclease free water up to 25 μl (4.5 μl).

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer</th>
<th>Oligonucleotide sequence (5’-3’)</th>
<th>Annealing temp. °C</th>
<th>Product size bp</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>16srRNA</td>
<td>16srRNA-F</td>
<td>5’-TTTAAGCGAGGAGGAGGAGG-3’</td>
<td>58°C for 30 sec.</td>
<td>240</td>
<td>(11)</td>
</tr>
<tr>
<td>16srRNA</td>
<td>16srRNA-R</td>
<td>5’-ATTCTACCATCCTGATCCC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>gltA</td>
<td>gltA-F</td>
<td>5’-AAATTTACAGTGACATGATGCAC-3’</td>
<td>55°C for 1 min.</td>
<td>722</td>
<td>(12)</td>
</tr>
<tr>
<td>gltA</td>
<td>gltA-R</td>
<td>5’-CCAGAGGATACGCAGAGATAC-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>RecA</td>
<td>RecA-F</td>
<td>5’-CACCGCCGTAAGAATGCATTA-3’</td>
<td>54°C for 30 sec.</td>
<td>425</td>
<td>(24)</td>
</tr>
<tr>
<td>RecA</td>
<td>RecA-R</td>
<td>5’-AAGGAGCCTCAGGGTATA-3’</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Results:**

On CHROMagar Acinetobacter species appeared as bright salmon-red colonies at 24 hours. Colony morphology of MDR and non-MDR Acinetobacter was indistinguishable on CHROMagar Acinetobacter. CHROMagar Acinetobacter also allowed growth of other red colonies. These red colonies were identified as
other gram-negative bacteria other than Acinetobacter and were distinguishable from Acinetobacter colonies by variation of their red color, colony morphology and oxidase reaction. Eight isolates appeared as shiny red colonies, tested oxidase-positive and were identified as five *Pseudomonaspseudoalcaligenes* and three isolates Moraxella group and on CHROMagar orientation Acinetobacter spp. Appeared as Creamy color, while other gram-negative bacteria showed blue color all summarized in the Figure 1 and Table 2.

![Figure 1: Cultured bacteria view in CHROMagar Acinetobacter and CHROMagar orientation after 24 hr](image)

**Table 2: Comparison of CHROMagar Acinetobacter and CHROMagar orientation for selection *A. Baumannii***

<table>
<thead>
<tr>
<th>Isolate</th>
<th>No.</th>
<th>CH. Acinetobacter</th>
<th>Orientation CH. Acinetobacter Color/colony</th>
<th>CH. Orientation color/colony</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter spp.</td>
<td>47</td>
<td><em>47</em></td>
<td>47</td>
<td>*bright salmon -Red/convex</td>
</tr>
<tr>
<td>Other gram-negative Bacteria</td>
<td>8</td>
<td><em>8</em></td>
<td>8</td>
<td>blue/convex</td>
</tr>
<tr>
<td>Total</td>
<td>55</td>
<td>55</td>
<td>55</td>
<td>55</td>
</tr>
</tbody>
</table>

*Small colonies, CH.Acinetobacter:CHROMagar Acinetobacter CH. Orientation:CHROM orientation*

In Vitek-2 compact system identification to species level. Out of the 55 strains, 43 strains were isolated from sputum, 5 from wound infections, and 7 from urine. The VITEK-2 compact system identified all the strains with a level of 95-99% probability (Table 3). 45 strains of *Acinetobacter baumannii* complex were identified with level 99%. 5 strains *Pseudomonaspseudoalcaligenes* with level 99%. 3 Moraxella group strains of probability 95%, and 2 strains *Acinetobacterradioresistans* with level 99%. all summarized in the Table 3.

In PCR our result showed all genes were found to be present in all 45 (100%) *A. baumannii* clinical studied isolates and take time 3:00 hr. to completed for each gene. The results of the presence of 16srRNA, gIIA and RecA genes are exemplified by the isolates shown in (Figure 3, 4, 5).
Table 3: results of identification all isolates by Vitek 2 compact system.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>No.</th>
<th>Probability of Id %</th>
<th>Time of Id inhr.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acinetobacter baumannii</td>
<td>45</td>
<td>99%</td>
<td>6:00 hr.</td>
</tr>
<tr>
<td>Acinetobacter radioresistans</td>
<td>2</td>
<td>99%</td>
<td>8:00 hr.</td>
</tr>
<tr>
<td>Moraxella group</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moraxella lacunata</td>
<td>3</td>
<td>95%</td>
<td>10:00 hr.</td>
</tr>
<tr>
<td>Moraxella nonliquefaciens</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moraxella osloensis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pseudomonas pseudoalcaligenes</td>
<td>5</td>
<td>99%</td>
<td>10:00 hr.</td>
</tr>
<tr>
<td>Total isolates</td>
<td>55</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Id = Identification, No. = Number of isolates

Fig. 3: Gel electrophoresis (1% agarose, 100 V/cm for 1hr. stained with ethidium bromide) for detect 16srRNA gene for some *A. baumannii* isolates. Line M 100bp DNA ladder, lines (1-12) positive results with 240bp amplicon.

Fig. 4: Gel electrophoresis (1% agarose, 100 V/cm for 1hr., stained with ethidium bromide) for detect gltA gene for some *A. baumannii* isolates. Line M 100bp DNA ladder, lines (1,2,3,4,5,6,7,8,9,10, 11) positive results with 722bp amplicon.

Fig. 5: Gel electrophoresis (1% agarose, 100 V/cm for 1hr., stained with ethidium bromide) for detect RecA gene for some *A. baumannii* isolateLine M 100bp DNA ladder, lines (1,2,3,4,5,6,7, 8) positive results with 425bp amplicon.
**Discussion:**

Our study results showed that CHROMagarAcinetobacter was 100% sensitive for both MDR and non-MDR-Acinetobacter when compared to PCR but their study reported that CHROMagarAcinetobacter was both sensitive (91.7%) and specific (89.7%) for MDR-*A. baumannii* when compared to PCR also was an investigational culture media different from the one evaluated in our study. Their CHROMagarAcinetobacter contained agent that inhibits the growth of most gram-positive bacteria as well as carbapenem susceptible gram-negative bacteria while allowing Acinetobacter to appear as aqua blue colonies instead of the red colonies in our study [14]. While other study showed the cost of CHROMagarAcinetobacter was less than the cost of the molecular method, it had a comparable outcome, detecting all MDR isolates (*Acinetobacter spp.*, and others) with no missed cases[15]. When compared to CHROMagar orientation, Acinetobacter species appeared as bright salmon red colonies on CHROMagarAcinetobacter facilitating easier identification and isolation. However, CHROMagarAcinetobacter did not differentiate MDR from non-MDR-Acinetobacter and further susceptibility testing is needed to confirm multidrug resistance status. In addition, CHROMagarAcinetobacter allows growth of red colonies that were identified as other gram-negative bacteria besides Acinetobacter; however, these other bacteria could be distinguished from Acinetobacter by variation of their red color, colony morphology and oxidase except [16]. In other studies by [20] have proved an efficiency of VITEK-2 System with 95-99% probability of accurate identification of all strains. This corresponds with our results, but in earlier study conducted by [21] results showed that the vast majority of isolates belong to *A. calcoaceticus A. baumannii complex* (93.8%), but in our study we found majority of isolate was *A. baumannii complex* (81.8%). This may be due to the difference in increasing levels of capacity and automation[22]. The early diagnosis of *Abbaumannii* with prompt application of appropriate therapy may prevent systemic involvement. The time required for the identification of *A. baumannii* based on culture methods is at least 18 hr. Table4 were recognized by the VITEK-2 framework as *A. baumannii* this outcome ought to be seen as an impediment of the phenotypic test, as opposed to the instability of VITEK-2 distinguishing proof results, since the phenotypic test can't separate microorganisms having a place with the ACB complex. The majority of the VITEK - 2 hardware utilized as a part of healing centers is really set up in a manner that when an animal types having a place with the ACB complex is recognized, it is naturally assigned as *A. baumannii.* [25].

The stamped contrast of the precision rates of the VITEK - 2 framework in distinguishing Acinetobacter was found by test bunch. The exactness rates of the VITEK - 2 framework in distinguishing Acinetobacter clinical disconnects were 76.6% in the test bunch in study reported by [25] while in our study was 81.8% that near our consequence of the test bunch Table 4.

The main differences of these groups are the sample size. The proportion of *A. baumannii* may explain the difference of the accuracy rates because the performance of VITEK- 2 system for *A. baumannii* is good but not good for other strains. Since bacteria belonging to the non-ACB complex are rare, obtaining a sufficient number of isolates to study the characteristic features of each species is difficult. For further study of the clinical characteristics, pattern of resistance to antibiotics, and distribution of genes of each bacterial species, an sample number of strains of each species must be collected and identified by molecular biological methods.

PCR is a method that combines rapid nucleic acid extraction, amplification, and detection. The time for microbiological identification using PCR can be as short as 3- 4 hrs, which greatly improves the turnaround time. The application of PCR was limited by the low positive predictive value, which might be because of the following possibilities. First, negative culture results could have been obscured by prior antimicrobial therapy, which was applied when patient was first suspected. As such, the dead bacterial remnants would fail to grow on culture plates but could be detected by PCR. Second, the more sensitive nature of PCR detection might lead to the detection of a small number of *Abaumannii*, which might not be detected in culture or be outgrown by the other bacteria present in the specimens. Third, the more sensitive PCR method might detect a small number of *A baumannii*, that is, however, not the responsible agent for the infection. [23]. From the Table 4 it is clear
from the results indicated in there is a consensus expressed as agreement % in the diagnostic accuracy by using PCR and Chromagar techniques.

<table>
<thead>
<tr>
<th>Method of diagnosis</th>
<th>Time</th>
<th>Cost</th>
<th>Sensitive</th>
<th>No. of isolates</th>
<th>Colony of bacteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chromagar</td>
<td>18-24 hr</td>
<td>Highly</td>
<td>100%</td>
<td>47</td>
<td>Live bacteria</td>
</tr>
<tr>
<td>Vitek 2 system</td>
<td>6-8 hr</td>
<td>Moderate</td>
<td>81.8%</td>
<td>45</td>
<td>Live bacteria</td>
</tr>
<tr>
<td>PCR</td>
<td>3-4 hr</td>
<td>Cheap</td>
<td>100%</td>
<td>45</td>
<td>Dead bacteria</td>
</tr>
</tbody>
</table>

Table 4: Comparison between methods of diagnosis of Acinetobacterbaumannii

<table>
<thead>
<tr>
<th>Agreement %</th>
<th>Chromagar vs Vitek 2 system</th>
<th>Chromagar vs PCR</th>
<th>Vitek 2 system vs PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>86%</td>
<td>100%</td>
<td>86%</td>
</tr>
</tbody>
</table>

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REFERENCES


