

Rapid identification of nosocomial *Acinetobacter baumannii* isolated from a surgical intensive care unit in Egypt

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BACKGROUND: The rapid and accurate identification of nosocomial clinical isolates is the first essential step in investigating nosocomial outbreaks. We aimed to evaluate the performance of MDR-CHROMagar *Acinetobacter* versus matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in rapid detection of nosocomial *Acinetobacter baumannii* isolated from patients admitted to the surgical intensive care unit (SICU) of Kasr Alainy- Cairo University .

METHODS: Over a period of 9 months from January 2014 until September 2014, 234 samples were collected. All samples were directly cultured on MDR-CHROMagar *Acinetobacter* media. MALDI-TOF MS was used to identify all non-lactose fermenting colonies on conventional media. Confirmation of species identification was done by detecting the *blaOXA-51* like gene by PCR.

RESULTS: Statistical evaluation of MDR-CHROMagar *Acinetobacter* against *blaOXA-51* like PCR as the reference method for identification of *A baumannii* showed a sensitivity of 100% (95% confidence interval [CI]: 93.36% to 100%), specificity 98.8% (95% confidence interval [CI]: 96.04% to 99.68%), positive predictive value 96.4% (95%CI: 86.61% to 99.37%), negative predictive value 100% (95% CI: 97.36% to 100%). The statistical evaluation of MALDI-TOF against *blaOXA-51* PCR was 100% concordance.

CONCLUSION: MALDI-TOF MS was more specific than CHROMagar in identifying *Acinetobacter spp* and allowed further identification of non-*A baumannii* species such as *A hemolyticus* and *A nosocomialis*, which are less common *Acinetobacter spp* involved in hospital-acquired infections.

A *cinetobacter baumannii* is an emerging nosocomial pathogen that contributes to increased morbidity and mortality in infected patients, especially in intensive care units.^{1,2} The rapid and accurate identification of nosocomial clinical isolates is the first essential step in investigating outbreaks for proper and timely intervention in infection control programs.³ The molecular identification of *A baumannii* has been validated using *blaOXA-51*-like genes in previous studies. It is a simple and reliable way to detect variants from isolates of *A baumannii*.⁴

Matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) has been evaluated for identification of *Acinetobacter baumannii*.^{5,6} On the other hand many chromogenic media

are commercially available and are commonly used as a cheap and rapid detection method for organisms causing hospital-acquired infections.⁷⁻¹⁰

The aim of this study was to evaluate the performance of MDR-CHROMagar *Acinetobacter* versus matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) in rapid detection of nosocomial *A baumannii* isolated from patients admitted in Kasr Alainy- Cairo University- surgical intensive care unit (SICU).

METHODS

Over a period of 9 months from January 2014 until September 2014, 234 different samples were collected from patients who were admitted to surgical inten-

sive care unit (SICU) of Kasr Alainy tertiary hospital (Cairo University).

Identification of clinical isolates by (MALDI-TOF MS)

All samples were cultured on different media; blood, chocolate and MacConkey agars (Oxoid Ltd, Basingstoke, Hampshire, England). Non-lactose fermenting (NLF) colonies were identified by conventional biochemical testing and antimicrobial susceptibility testing using the disc diffusion method (Modified Kirby Bauer technique) on Mueller-Hinton agar according to Clinical and Laboratory Standards Institute¹¹ guidelines. Further identification was done by MALDI-TOF MS on a Microflex LT instrument (Bruker Daltonics GmbH, Leipzig, Germany) with FlexControl (version 3.0) software (Bruker Daltonics) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2 to 20 kDa, according to the instructions of the manufacturer. For isolate identification, the row spectra were compared with those in the Biotyper 56 database and a log (score) of ≥ 2 was considered for a secure species identification. Spectra were acquired with a microflex LT mass spectrometer (Bruker Daltonics), recorded in the positive linear mode at a laser frequency of 20 Hz, ion source 1 voltage 59 20 kV, ion source 2 voltage 8.5 kV, and a mass range from 2000 to 20 000 kDa, as described elsewhere.¹² For MALDI-TOF MS, bacteria were cultured for 24h at 37°C on MacConkey agar and extracts were prepared as described previously.^{12,13}

Identification of clinical isolates by MDR-CHROMagar Acinetobacter Media

All samples were directly inoculated on the MDR-CHROMagar *Acinetobacter* Media (CHROMagar, France) according to the manufactures recommendations. MDR-CHROMagar *Acinetobacter* is used to detect different types of multidrug resistant (MDR) organisms.

Identification of clinical isolates by blaOXA-51 like gene

Molecular analysis by conventional PCR was done using the blaOXA-51 -like gene as described by Turton et al, 2006. DNA was extracted by the heating method of Vaneechoutte; briefly, strains were grown on blood agar plates and were incubated overnight at 37°C to isolate single colonies. After incubation, four or five discrete colonies of each strain were resuspended in 50 μ L of sterile distilled water in a 0.5-mL Eppendorf tube. To lyse the cells and extract DNA, the tubes were heated for 10 min at 95°C, then cooled on ice, and were centri-

fuged in a microcentrifuge (Microfuge Lite; Beckman) at 12 000 rpm for 30 sec to remove the cell debris. These crude DNA extracts were kept on ice for immediate use or frozen at -20°C until the rest of the PCR reagents were prepared.^{14,15}

DNA amplification was performed in 25- μ L reaction volumes with 3 μ L of extracted DNA, 12.5 pmol of each primer (OXA-51-likeF 5'-TAA TGC TTT GAT CGG CCT TG-3', OXA-51-likeR 5'-TGG ATT GCA CTT CAT CTT GG-3') (Bioneer, AccuOligo), and 1.5 U of Taq DNA polymerase in 1 μ L PCR buffer containing 1.5 mM MgCl₂ (QIAGEN, inc., Chatsworth, CA, USA) and 200 μ M of each dTPs. Conditions of the thermal cycler (Primus 25 advanced- PEQ LAB – GmbH-V1106E Germany) for PCR was the following: 94°C for 3 min, and then 35 cycles at 94°C for 45 s, 57°C for 45 s, and 60°C for 1 min, followed by a final extension at 72°C for 5 min.⁸

The amplified products were detected by agarose gel electrophoresis using 1.5% agarose (BIORAD), stained with ethidium bromide and examined by UV (ultraviolet) transillumination (Sigma). Positive blaOXA-51 like fragment was 369 bp.

RESULTS

From all 234 samples inoculated on the MDR-CHROMagar *Acinetobacter*, 59 isolates grew on the media, 56/234 isolates showed the typical morphology of MDR *Acinetobacter* spp appearing as red colonies, while 3 samples revealed different colored colonies; two isolates showed black colonies (further identified as *Proteus* spp.) and one isolate showed green colonies (further identified as *K pneumoniae*). (Figures 1, 2 and 3).

Molecular identification of the 56 MDR *Acinetobacter* spp recovered from MDR-CHROMagar *Acinetobacter* was done by blaOXA-51 like PCR, which was positive in 54/56 (96.4%) of MDR *Acinetobacter* spp. isolates, identifying them as *Acinetobacter baumannii* (Figure 4). Using MALDI TOF MS, the 56 isolates were identified as *Acinetobacter* species with a score value >2.7, 54 identified as *Acinetobacter baumannii*, one as *Acinetobacter haemolyticus* and another one as *Acinetobacter nosocomialis*.

Statistical evaluation of MDR-CHROMagar *Acinetobacter* against blaOXA-51 like PCR as the reference method for identification of *Acinetobacter baumannii*, showed a sensitivity 100% (95% confidence interval [CI]: 93.36% to 100%), specificity 98.8% (95%CI: 96.04% to 99.68%), positive predictive value 96.4% (95% CI: 86.61% to 99.37%), negative predictive value 100% (95% CI: 97.36% to 100%). The statistical evalu-



Figure 1. CHROMagar MDR *Acinetobacter* agar plate showing typical red colonies of MDR *Acinetobacter* spp.



Figure 3. CHROMagar MDR *Acinetobacter* agar plate showing MDR *Klebsiella pneumoniae* with green-colored colonies.



Figure 2. CHROMagar MDR *Acinetobacter* agar plate showing MDR *Proteus mirabilis* with black-colored colonies.

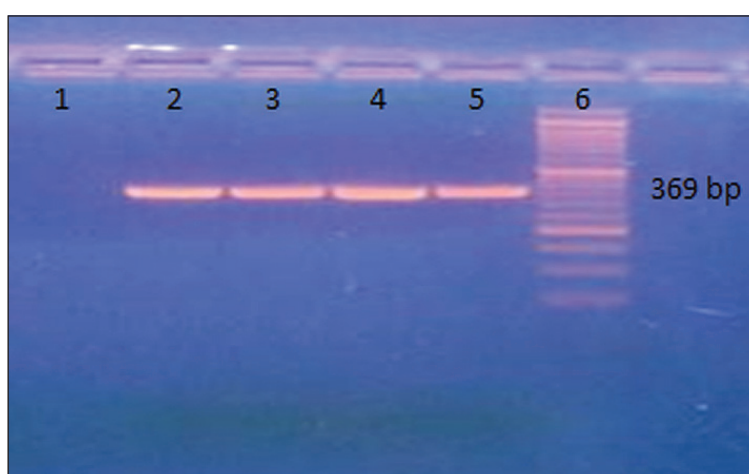


Figure 4. Lane (1) is negative for *blaOXA51*, Lanes (2), (3), (4) and (5) are *OXA51* positive while Lane (6) is the ladder lane.

ation of MALDI TOF against *blaOXA-51* like gene PCR showed 100% concordance.

DISCUSSION

In our study, we evaluated the performance of MDR CHROMagar *Acinetobacter* against reference methods for early and accurate identification of MDR *Acinetobacter baumannii*. We found that it was 96.4% sensitive and 100% specific for MDR *Acinetobacter baumannii* when compared to *blaOXA-51* like PCR as the reference method for identification of *Acinetobacter baumannii*. *Acinetobacter* spp appeared as bright salmon red colonies, while the other MDR gram-negative bacteria appeared in different colors. Limitations of CHROMagar are as follows: some

Enterobacteriaceae strains may grow as blue to metallic blue colonies and in the presence of the MDR selective supplement these strains should be considered as potentially harboring multi-drug resistance.

Other non-fermenter gram-negative strains such as *Pseudomonas* spp or *Stenotrophomonas* spp can have an appearance similar to *Acinetobacter* and are frequently MDR, and can grow in the presence of the MDR-selective supplement. Fortunately, *Pseudomonas* strains can be easily differentiated by an oxidase test, while *Stenotrophomonas* strains can be distinguished as forming tiny colonies at 18-24 hours as stated in the CHROMagar manufacturer insert.

Wareham et al 2011 also reported that *S maltophilia* grew on CHROMagar *Acinetobacter* and had a

red color similar to *A baumannii*, making it difficult to distinguish.¹⁵

However, MDR CHROMagar *Acinetobacter* may save time in the absence of a red color, indicating the absence of MDR *Acinetobacter* species in patient samples by screening, leading to improvement in infection control strategies and stopping the use of empirical antibiotics in screen negative patients.

Various studies have found results similar to ours such as Ajao et al 2011, who found that CHROMagar *Acinetobacter* was 100% sensitive for *Acinetobacter* isolates compared to MacConkey agar, which was 89% sensitive for *Acinetobacter* species and 91% sensitive for MDR-*A baumannii*. Helal et al found that MDR *Acinetobacter* CHROMagar was 92.9% sensitive and 100% specific for MDR *Acinetobacter baumannii*.^{16,17}

In another study that evaluated the use of MDR-CHROMagar *Acinetobacter* for detection of MDR *Acinetobacter* species in nasal and rectal surveillance cultures, there was a 100% concordance between CHROMagar *Acinetobacter* and Vitek 2 system (bioMerieux) in terms of identification; they had the same results in antimicrobial susceptibility using E-test strips (bioMerieux, Marcy-l'Étoile, France).¹⁸

The ubiquitous nature of *bla*OXA-51-like genes in *A baumannii* has led to this gene becoming an important genetic marker in identification of the organism to the species level. Polymerase chain reaction (PCR) is the optimal tool for the identification of OXA-type carbapenemases, though carries the disadvantages of higher cost and the requirement for trained personnel.¹⁹

Molecular identification of the 56 MDR *Acinetobacter* isolates recovered from MDR CHROMagar *Acinetobacter* was done by PCR for detection of *oxa-51* like gene, which was positive in 96.4% (54/56) MDR *Acinetobacter*, identifying these 54 cases as *A baumannii*, with only 2 cases of MDR non-*baumannii* spp.

A study performed by Gordon and Wareham reported that CHROMagar *Acinetobacter* was both sensitive (91.7%) and specific (89.7%) for MDR-*A baumannii* when compared to PCR. Investigators in Korea reported that all their isolates (28/28) identified as *A baumannii* were *bla*OXA-51-like positive.^{20,21} While the cost of CHROMagar *Acinetobacter* 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.

Many studies have reported having confidence in MALDI-TOF MS for different microorganisms.

Most of these studies included strains from reference and culture collections. The application of MALDI-TOF MS in routine microbiology laboratories in many countries is now expanding as it has the advantage of identification of microorganisms in minutes versus conventional biochemical testing that takes 24 hours or even more.^{19,21}

For *A baumannii*, the manufacturer of Bruker MALDI-TOF MS has reported that species of the genus *Acinetobacter* have very similar patterns; therefore, distinguishing species is difficult. However, confident results can be obtained with duplicate identification of each isolate while relying on a more stringent spectra score of >2.3 ("highly probable species identification") instead of >2 ("secure genus identification, probable species identification") as stated by the Bruker MALDI Biotyper software.

A baumannii is the most clinically important species in hospital settings that we could detect in our study using *oxa-51* like PCR. MALDI Biotyper system allowed further identification of non-*Baumannii* species as *A hemolyticus* and *A nosocomialis*, which are less common *Acinetobacter* species involved in hospital-acquired infections. The obstacle with the MALDI Biotyper is the initial cost of the system, but the cost per test for isolate identification is low compared to conventional biochemical tests and PCR analysis. However, in epidemiological settings, the use of MDR CHROMagar *Acinetobacter* will be more cost and labor effective, as there will be a quick answer for the presence or absence of infection with an MDR organism. Also, there will be no need for performing separate antimicrobial susceptibility testing that must follow identification using MALDI Biotyper or PCR.

In conclusion, the MDR CHROMagar *Acinetobacter* is a cost and labor effective method for detection of MDR *Acinetobacter* spp in hospital settings that can be used with confidence (94.6%) when compared to *oxa-51*-like PCR, the gold standard for *A baumannii* identification, and the MALDI-TOF system. The results suggest that MALDI-TOF MS can be used for real-time detection of *Acinetobacter* outbreaks, before results from DNA-based systems are available.

Conflict of interest

None declared.

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