IRIS AperTO

UNIVERSITÀ DEGLI STUDI DI TORINO

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Evaluation of the Amplex eazyplex SuperBug Acineto test for direct detection of multidrug-resistant Acinetobacter baumannii bloodstream infections in high endemicity settings [S. Comini is the corresponding author]

This is a pre print version of the following article:

Original Citation:

Availability:

This version is available http://hdl.handle.net/2318/1818461 since 2023-02-04T12:42:56Z

Published version:

DOI:10.1016/j.jhin.2021.09.015

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

15 January 2025

Evaluation of the Amplex eazyplex SuperBug Acineto test for direct detection of multi-drug resistant *Acinetobacter baumannii* bloodstream infections in high endemicity settings

Sara Comini[1]*, Gabriele Bianco[2]*, Matteo Boattini[2], Marco Iannaccone[2], Roberto Casale[2], Giuliana Banche[1], Rossana Cavallo[1,2], Cristina Costa[1,2]

[1]Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy.

[2]Microbiology and Virology Unit, University Hospital Città della Salute e della Scienza di Torino, Turin, Italy

*These authors contributed equally to this work

CORRESPONDING AUTHOR: Sara Comini

Department of Public Health and Pediatric Sciences, University of Turin, Turin, Italy.

E-mail: sara.comini@unito.it

Tel: +390116705627

Dear Sir,

Acinetobacter genus comprises many closely related species, including those belonging to the *Acinetobacter calcoaceticus-baumannii* (ACB) complex (e.g., *A. baumannii, A. nosocomialis, A. pittii,* and *A. calcoaceticus*), which are frequently misidentified using the traditional techniques employed in clinical microbiology. The ACB complex is most commonly associated with life-threatening infections such as ventilator-associated pneumonia, meningitis and sepsis and is often involved in outbreaks in healthcare facilities given its ability to survive on dry surfaces [1].

Carbapenem-resistant *A. baumannii* (CRAB) has emerged globally, and has been recognized as a matter of major concern given its multidrug resistant (MDR) phenotype and the limited number of treatment options. OXA-type carbapenemases, most notably the OXA-23, OXA-24/-40 and OXA-58 groups, represent the main mechanisms involved in carbapenems resistance. Other acquired carbapenemases identified in *Acinetobacter* spp. include class B metallo β-lactamases (e.g. NDM, VIM and IMP) and class A carbapenemases (e.g. KPC and GES variants) [1,2].

The Amplex eazyplex® SuperBug Acineto (AmplexDiagnostics GmbH, Gars am Inn, Germany) test is a commercial loop-mediated isothermal amplification (LAMP) assay detecting *A. baumannii* species-specific blaOXA-51 gene and acquired carbapenemases blaOXA-23-like (blaOXA-23,-27, -49, -73), blaOXA-24/-40-like (blaOXA-24, -40, -25, -26, -72), blaOXA-58-like (blaOXA-58, -96, -97) and blaNDM (blaNDM-19,-16). The SuperBug Acineto kit is CE-marked, validated for isolated bacterial colonies and showed 100% concordance with traditional PCR assays or Whole Genome Sequencing [3-5].

Here we evaluated the Amplex eazyplex® LAMP assay to detect *A. baumannii* and its main acquired carbapenemases directly from routine blood culture (BC) bottles. This one-year (August 2020-August 2021) prospective study included positive BCs showing Gram-negative rods at microscope observation recovered from patients both admitted in COVID-19 Intensive Care (ICU) and Burn

Units and presenting with rectal or respiratory carriage of MDR *A. baumannii* in the 30 days preceding the BSI event. Only one positive BC bottle per patient was tested.

Positive BC bottles (BACT/ALERT Plus, BioMérieux, Marcy l'Ètoile, France) on BactAlert Virtuo (BioMérieux, Marcy l'Ètoile, France) were submitted to routine diagnostics which included direct MALDI-TOF MS analysis directly from BC fluid, and microbial identification toghether with antimicrobial susceptibility testing on overnight subculture, as previously described [4]. In parallel, an aliquot was prospectively tested using the LAMP assay and results were compared.

Briefly, 25 µl of BC fluid were mixed with 500 µl of RALF provided by the LAMP kit, and boiled for 2 min. After centrifugation at 1000 g for 30 seconds, 25 µl of the supernatant were added to each tub of the eazyplex® test strip containing the lyophilized master mix. The strip was gently knocked to remove air bubbles and loaded into the Genie II instrument. Amplification was measured by real-time fluorescence detection using a DNA intercalating dye.

During the study period, 63 Gram-negative positive BCs, acquired from 42 COVID-19 ICU and 21 burn patients, respectively, met the study inclusion criteria. Of these, 24 out of 63 (38.1%) patients presented with KPC-producing *K.pneumoniae* rectal colonization. Microbiological results obtained by conventional and LAMP eazyplex® assay were summarized in Table 1. Overall, 41 out of 63 (65.1%) of Gram-negative BSIs were sustained by CR-ACB. Among these, 17.1% (7 out of 41) were polimicrobial, involving *Enterobacterales*, *Pseudomonas*, *Enterococcus* and *Candida* species. BCs not involving CR-ACB were 22 (34.9%); among these, a carbapenems susceptible *A. Baumannii* was isolated in one sample. KPC-producing *K.pneumoniae* was isolated in 15 (23.8%) of BC samples, of which three in co-infection with CR-ACB.

MALDI-TOF MS analysis showed poor performance in identifying *A. baumannii*, above all in polimicrobial BCs, as also previously reported [6].

Excluding one BC sample with invalid result, the Amplex eazyplex® SuperBug Acineto test showed a 100% concordance with conventional phenotypic results, identifying all bloodstream infections

(BSIs) sustained by OXA-23-producing CR-ACB, carbapenems susceptible *A. baumannii* and providing no false positive/negative result in patents with BSI sustained by other bacterial species.

In the era of MDR Gram-negative infections, integration of rapid diagnostics together with antimicrobial stewardship interventions are the key to timely support transition to effective therapy and implement infection control measures [7,8]. Results from this study highlight that Amplex eazyplex® SuperBug Acineto test can detect *A. baumannii* and the main acquired carbapenemases associated with its carbapenems resistance. The rapid and simple setting-up, requiring minimal hands-on time make this test suitable for implementation in settings with high endemicity of CR-ACB, such as ICU and Burn Unit patients. Moreover, as also previously observed for carbapenemases-producing *Enterobacterales* [6], CR-ACB carriage represents a relevant risk factor for subsequent CR-ACB BSI, suggesting that patients with CR-ACB may represent a selected patient cohort in which rapid testing results might have a higher clinical impact in term of antibiotic management and cost-effectiveness.

In conclusion, to our knowledge, this study represents the first evaluation of a LAMP assay,the Amplex eazyplex® SuperBug Acineto test, to identify in less than 30 minutes *A. baumannii* and its main acquired carbapenemases directly from BC bottles.

Monocenter design of our study represents the main limitation. Further worldwide investigations on a wider spectrum of OXA-type and the other classes of carbapenemases are warranted to strengthen the generalizability of our results. Further studies are also needed to assess the impact of this molecular assay on antimicrobial stewardship, clinical outcomes and infection control programmes.

FUNDING

This work was supported by internal funding.

CONFLIT OF INTEREST:

The authors declare that they have no conflict of interest.

ETHICAL APPROVAL:

The study was conducted in accordance with the ethical standards and Helsinki Declaration and approved by the Institutional Review Board.

REFERENCES

1. Nasr P. Genetics, epidemiology, and clinical manifestations of multidrug-resistant Acinetobacter baumannii. J Hosp Infect 2020 ;104:4-11. doi: 10.1016/j.jhin.2019.09.021.

2. Poirel L, Nordmann P. Carbapenem resistance in Acinetobacter baumannii: mechanisms and epidemiology. Clin Microbiol Infect 2006;12:826-36. doi: 10.1111/j.1469-0691.2006.

3. Vergara A, Zboromyrska Y, Mosqueda N, Morosini MI, García-Fernández S, Roca I, et al. Evaluation of a loop-mediated isothermal amplification-based methodology to detect carbapenemase carriage in Acinetobacter clinical isolates. Antimicrob Agents Chemother 2014;58:7538-40. doi: 10.1128/AAC.03870-14.

4. Jakovac S, Goić-Barišić I, Pirija M, Kovačić A, Hrenović J, Petrović T, et al. Molecular Characterization and Survival of Carbapenem-Resistant *Acinetobacter baumannii* Isolated from Hospitalized Patients in Mostar, Bosnia and Herzegovina. Microb Drug Resist 2021;27:383-90. doi: 10.1089/mdr.2020.0163.

5. Haldorsen BC, Janice J, Samuelsen Ø. Evaluation of the Amplex eazyplex® SuperBug Acineto test for detection of acquired OXA and NDM carbapenemases in Acinetobacter spp. J Glob Antimicrob Resist 2021 ;24:340-1. doi: 10.1016/j.jgar.2021.01.019

6. Bianco G, Boattini M, Iannaccone M, Pastrone L, Bondi A, Peradotto M, et al. Integrating rapid diagnostics in Gram-negative bloodstream infections of patients colonized by carbapenemase-producing Enterobacterales. J Hosp Infect 2021;110:84-8. doi: 10.1016/j.jhin.2021.01.015.

7. Bianco G, Boattini M, Iannaccone M, Sidoti F, Cavallo R, Costa C. Detection of antibiotic resistance genes from blood cultures: performance assessment and potential impact on antibiotic therapy management. J Hosp Infect 2019;102:465-9. doi: 10.1016/j.jhin.2019.03.007.

8. Boattini M, Bianco G, Iannaccone M, Zanotto E, Sidoti F, Almeida A, et al. Detection of Carbapenemase and CTX-M Encoding Genes Directly from Bronchoalveolar Lavage Using the CRE and ESBL ELITe MGB Assays: Toward Early and Optimal Antibiotic Therapy Management of Critically Ill Patients with Pneumonia. Microb Drug Resist 2021;27:241-6. doi: 10.1089/mdr.2020.0199.