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Resist Acineto rapid immunological test for the detection of acquired carbapenemase producers among *Acinetobacter* spp

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ABSTRACT

The Resist Acineto from Coris Bioconcept is a novel immunochromatographic test for detection of the major acquired carbapenemases (OXA-23, OXA-40, OXA-58, and NDM) identified in *Acinetobacter* spp. This rapid and easy-to-perform test showed an excellent specificity and sensitivity, with positive and negatives predictive values of 100% in both cases.

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The increasing global prevalence of carbapenem-resistant *Acinetobacter* spp. represents a significant threat to public health [1]. In Acinetobacter baumannii, which is the most important *Acinetobacter* genospecies associated with human infections, resistance to carbapenems is almost always associated to the production of acquired carbapenem-hydrolysing class D β-lactamases (oxacillinases) of groups OXA-23, OXA-24/-40, and OXA-58. Less frequently, resistance to carbapenems may be also due to the acquisition of carbapenem-hydrolysing metallo-β-lactamases such as NDM-type enzymes, and even more rarely IMP- or VIM-type enzymes [2,3]. Therefore, a rapid and accurate identification of carbapenemase producers in patients infected or colonized is of critical importance for both treating infected patients and infection control purposes. Indeed, and by contrast to the situation observed in Enterobacterales, identification of a carbapenemase production in *A. baumannii* systematically indicates that the positive isolate is resistant to carbapenems. Molecular based-detection of carbapenemase encoding genes is an interesting alternative but remains costly, time consuming, not easily implementable worldwide, and often only detects the most common carbapenemase-encoding genes [2–4]. Either the biochemical detection of the carbapenem hydrolysis or the immunological detection of the ß-lactamase proteins are widely-used testing methods for the rapid detection of carbapenemases, in particular in Enterobacterales, but they have not been implemented for *Acinetobacter* spp. [2–4].

For this reason, a novel immunochromatographic detection test, namely the Resist Acineto (Coris Bioconcept.), has been developed. This lateral flow assay is intended for the detection of the 4 main carbapenemase types found in *Acinetobacter* spp., including OXA-23, OXA-40/-58, and NDM-type enzymes. The present study was aimed to evaluate this test using a representative collection (n = 174) of well-characterized carbapenemase and noncarbapenemase producing isolates belonging to various *Acinetobacter* species recovered at the Swiss National Reference Center for Emerging Antibiotic Resistance (Table 1). This collection included 149 *A. baumannii*, 11 *A. pittii*, four *A. radioresistens*, three *A. ursingii*, two *A. calcoaceticus*, two *A. nosocomialis*, one *A. bereziniae*, one *A. lwoffii* and one *A. junii*.

The acquired carbapenemase types were distributed as follows: OXA-58 producers (n = 25), OXA-40 (n = 31), OXA-72 (n = 3), OXA-23 (n = 38), and NDM (n = 5). Additionally, 28 isolates coproducing 2 acquired carbapenemases were included (10 producing NDM + OXA-23, six OXA-23 + OXA-40, 8 NDM + OXA-40, and 4 NDM + OXA-58), those double carbapenemase producers being of particular relevance taking in account that they are increasingly identified worldwide (Table 1). All acquired carbapenemase genes were identified and confirmed by PCR and subsequent sequencing. Isolates were grown on Columbia with 5% Sheep blood agar plates (bioMérieux, Marcy l'Etoile, France) for 20 hours at 37°C.

The Resist Acineto test was performed according to the manufacturer's recommendations. Briefly, one calibrated 1 μl-loopful of the strain to be tested was resuspended in 6 drops of sample treatment

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Table 1
Results of the Resist Acineto assay.

Species (no.)	Resistance determinant(s) (no.)	RESIST ACINETO assay
OXA-58 (n = 25)		
Acinetobacter baumannii	OXA-58 (n = 24)	OXA-40/58
Acinetobacter bereziniae	OXA-58 (n = 1)	OXA-40/58
OXA-40 (n = 31)		
Acinetobacter baumannii	OXA-40 (n = 31)	OXA-40/58
OXA-72 (n = 3)		
Acinetobacter baumannii	OXA-72 (n = 3)	OXA-40/58
OXA-23 (n = 38)		
Acinetobacter baumannii	OXA-23 (n = 37)	OXA-23
Acinetobacter radioresistens	OXA-23 (n = 1)	OXA-23
NDM (n = 5)		
Acinetobacter baumannii	NDM-1(n = 3)	NDM
Acinetobacter baumannii	NDM-2 (n = 1)	NDM
Acinetobacter nosocomialis	NDM-1 (n = 1)	NDM
Double carbapenemase (n = 28)		
Acinetobacter baumannii	NDM-1 + OXA-23 (n = 9)	NDM + OXA-23
Acinetobacter baumannii	NDM-5 + OXA-23 (n = 1)	NDM + OXA-23
Acinetobacter baumannii	NDM-1 + OXA-40 (n = 8)	NDM + OXA-40/58
Acinetobacter baumannii	NDM-1 + OXA-58 (n = 2)	NDM + OXA-40/58
Acinetobacter baumannii	NDM-9 + OXA-58 (n = 1)	NDM + OXA-40/58
Acinetobacter baumannii	OXA-23 + OXA-40 (n = 6)	OXA-40/58 + OXA-23
Acinetobacter nosocomialis	NDM-1 + OXA-58 (n = 1)	NDM + OXA-40/58
Negative controls (n = 30)		
Acinetobacter baumannii	Wild type (n = 13)	Negatives
Acinetobacter baumannii	Overexpressed cephalosporinase (n = 5)	Negatives
Acinetobacter baumannii	GES-12 (n = 1)	Negative
Acinetobacter baumannii	GES-14 (n = 1)	Negative
Acinetobacter baumannii	IMP-5 (n = 1)	Negative
Acinetobacter baumannii	OXA-84 (n = 1)	Negative
Acinetobacter baumannii	PER-1 (n = 1)	Negative
Acinetobacter ursingii	Overexpressed cephalosporinase (n = 3)	Negatives
Acinetobacter calcoaceticus	Wild type (n = 1)	Negative
Acinetobacter calcoaceticus	TMB-1 (n = 1)	Negative
Acinetobacter junii	Wild type (n = 1)	Negative
Acinetobacter lfwoffii	OXA-134 (n = 1)	Negative
Unexpected results (n = 14)		
Acinetobacter pittii	Wild type (OXA-500) (n = 8)	OXA-40/58
Acinetobacter pittii	Wild type (OXA-564) (n = 2)	OXA-40/58
Acinetobacter pittii	NDM-1 and OXA-506 (n = 1)	OXA-40/58 + NDM
Acinetobacter radioresistens	OXA-813 (n = 2)	OXA-23
Acinetobacter radioresistens	OXA-103 (n = 1)	OXA-23

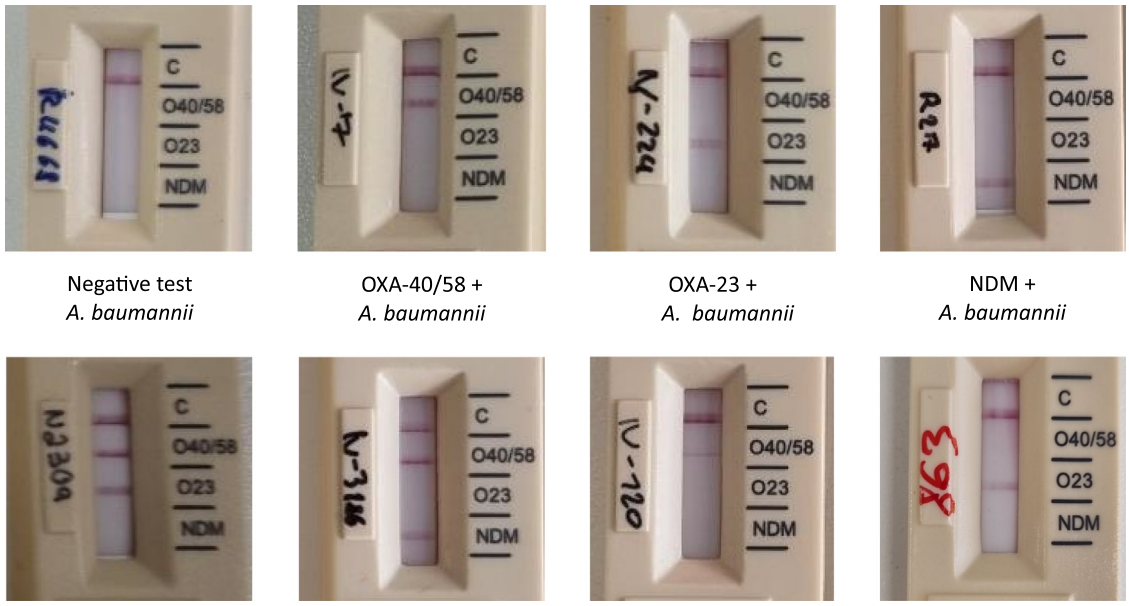

Fig. 1. Representation of RESIST ACINETO tests results.

solution included in the kit. Then, 100 μl of the lysis mixture was added into the sample well of the Resist Acineto cassette. The results were obtained within 15 minutes of incubation at room temperature. The Resist Acineto detected all acquired carbapenemases of the OXA-23-, OXA-40/OXA-58- and NDM-enzymes types (Table 1, Fig. 1). Noteworthy, all the 11 *A. pittii* strains tested gave positive results for the OXA-40/OXA-58 carbapenemases, and all the 4 *A. radioresistens* tested were positive for the OXA-23 carbapenemase. Detection of those enzymes respectively in *A. pittii* and *A. radioresistens* was actually expected considering that those 2 *Acinetobacter* spp. are known to possess intrinsic genes encoding either OXA-23-like enzymes for *A. radioresistens*, or OXA-213-like enzymes (showing significant identity with OXA-40) for *A. pittii* [5–7]. Identification of carbapenem-resistant *A. pittii* or *A. radioresistens* is rare in clinical settings. As an example, 4 out of 92 (4%) carbapenem-resistant *Acinetobacter* spp. were identified as belonging to those species in the collection of our National Reference Center in Switzerland (NARA) in 2022. It should however be underscored that phenotypic variations in term of carbapenem resistance have been reported in those *Acinetobacter* species, being related to variable expressions of those naturally-occurring ß-lactamase genes [8].

To confirm the cross-reactivity of the OXA-213-type enzymes with the OXA-40 antigenic test, an OXA-213-like encoding gene was cloned into plasmid pCR-BluntII-Topo (Invitrogen, Thermo Fisher) and transformed into *Escherichia coli* TOP10. This recombinant strain was then tested with the Resist Acineto test and gave a positive result at the level of the OXA-40 signal, confirming the false-positivity generated by such enzyme for *A. pittii* strains.

Noteworthy, the Resist Acineto test was successful in detecting all double carbapenemase producing isolates. The overall sensitivity and specificity were found to be 100% and 96%, respectively, and the positive and negative predictive values at 91% and 100%, respectively. After exclusion of the results obtained for the *A. pittii* and *A. radioresistens* isolates, no false positivity result was actually observed (Table 1). After exclusion of *A. pittii* and *A. radioresistens* results, sensitivity, specificity, positive and negative preductive values were 100%.

One limitation of the test is that it does not detect rare carbapenemases that may be found in *A. baumannii*, such as IMP- or VIM-type enzymes. An additional limitation of this test, as commonly observed with all similar immunochromatographic tests, is their ability to detect only known major enzymes compared to biochemical tests, previously proven to be sensitive for rare or unknown carbapenemase detection, as reported for the RapidAcineto Carba NP [9]. Further studies will be required to evaluate the accuracy of this Resist Acineto test using clinical samples (such as blood samples), which might constitute an interesting diagnostic tool.

In conclusion, the Resist Acineto is a rapid and easy-to-perform test, that showed an overall excellent specificity for detecting different variants of the 4 most common carbapenemases identified in *Acinetobacter* spp. Given its easiness of usage, its simplicity, and its short time-to-result, the Resist Acineto test is suitable for microbiology laboratories.

Authors' contribution

PN and LP designed the study. MB, SF, JF and AK performed the experiments. MB and PN drafted the manuscript. LP and PN wrote the final version of the manuscript. All authors agreed on the final version.

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Declaration of Competing Interest

PN and LP are inventors of the Rapid Acineto Carba NP test aimed to identify biochemically carbapenemase production in *Acinetobacter baumannii.*

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