UNIVERSIDADE FEDERAL DO RIO GRANDE DO SUL
FACULDADE DE FARMÁCIA
TRABALHO DE CONCLUSÃO DE CURSO DE FARMÁCIA

RAPID RESAIMIPENEM/ACINETOBACTER NP TEST: IS IT FEASIBLE AMONG *Pseudomonas aeruginosa*?

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Trabalho de Conclusão de Curso apresentado ao Curso de Farmácia da Universidade Federal do Rio grande do Sul como requisito à obtenção do título de grau de Farmacêutico.

Orientador: Prof. Dra. Juliana Caierão

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APRESENTAÇÃO

Esse Trabalho de Conclusão de Curso foi redigido sob a forma de artigo ao qual foi elaborado segundo as normas da revista Journal of Microbiological Methods, apresentadas em anexo.

Rapid ResaImipinem/Acinetobacter NP TEST: Is it feasible among *Pseudomonas aeruginosa*?

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A R T I C L E I N F O

Keywords:

antimicrobial resistance, carbapenemase, carbapenem-resistance, rapid detection

A B S T R A C T

Nonfermenting Gram negative bacilli, such as *Pseudomonas aeruginosa* and *Acinetobacter baumannii* are important nosocomial pathogens listed as critical among the World Health Organization priorities of pathogens which urgently require n e w antibiotics. The right empiric antibiotic prescription can contribute to a more successful treatment, seeing that these two known pathogen s are often found with resistance to antibiotics that are commonly used across the world. In this experiment, the use of a new method for detecting carbapenem-resistance in *Acinetobacter baumannii*, called the *Rapid ResaImipinem/ Acinetobacter* NP test, was tested if it could be applied also for *P. aeruginosa*. It was shown that exactly how it was projected for *A. baumannii* doesn't work for *P. aeruginosa* and it was suggested that testing further conditions, this technique might be implemented to detect *P. aeruginosa* resistance to carbapenems as well.

1. Introduction

Pseudomonas aeruginosa is one of the most frequent nosocomial opportunistic pathogens (1,2), commonly affecting hospitalized patients, especially those suffering with immunodeficiency, requiring ventilation, as well as patients with chronic obstructive pulmonary disease (COPD), among other predisposing conditions. These bacteria contributes to morbidity and mortality of patients with cystic fibrosis (3,4,5). Moreover, carbapenem resistance in *P. aeruginosa* and *Acinetobacter baumannii* was the main reason why both were listed in the "Priority 1: Critical" category of the World Health Organization (WHO) global priorities to which new antibiotics are urgently needed (6).

β-lactams are one of the most effective drugs against *P. aeruginosa*. They act inhibiting the peptidoglycan-assembling transpeptidases, avoiding adequate formation of cell wall. The carbapenem imipenem is one of the most effective drug against *P. aeruginosa*, however, the incidence of imipenem resistance is a major issue of concern. Carbapenem resistance among *P. aeruginosa* may include (i) derepression of the chromosomal AMPc cephalosporinase; (ii) production of carbapenemase belonging to class A, class D and/or class B; (iii) loss of outer membrane porin (OprD); (iv) overexpression of active efflux systems with a broad substrate profiles (1,6,7,8,9,10,11).

It is well recognized that there is a linear increased mortality associated with a first antibiotic administration delay in patients with severe septic shock, for example. In this concern, the early identification and treatment of infections with appropriate antibiotic therapy has an important impact on the outcome of patients with sepsis and septic shock (12). Therefore, an accurate and rapid method to detect carbapenem resistance is desirable to prescribe a more efficient treatment, rather than using an empirical treatment that might not work properly due to antibiotic resistance, which might be life threatening for patients. Indeed, the determination of minimum inhibitory concentrations (MICs) by the broth microdilution (BMD) has become unacceptably slow in the context of resistance to most antibiotics used as empiric therapy (13).

Rapid ResaImipenem/Acinetobacter NP test was developed to determine carbapenem susceptibility/resistance among *A. baumannii*. It is based on a colorimetric reaction, the reduction of resazurin to resorufin, by the metabolic activity of bacterial cells, detecting their growth in the presence of carbapenem (13). The main objective of this work was to evaluate the applicability of *Rapid ResaImipenem/ Acinetobacter* NP test among our collection of Acinetobacter spp, as well as *P. aeruginosa*, in detecting carbapenem resistance.

2. Materials and methods

2.1. Bacterial strains:

Overall, 9 *P. aeruginosa* and 55 *A. baumannii* recovered from two different hospitals of Porto Alegre, Brazil, were included in the study. Isolates were maintained at the Bacteriology Laboratory (LABAC) from the Federal University of Rio Grande do Sul, Brazil.

2.2 Susceptibility test:

Susceptibility test: Resistance to imipenem was defined by determining MIC values by broth microdilution in duplicate, interpreted according to EUCAST breakpoints (*P. aeruginosa* isolates were categorized as susceptible when MICs using imipenem were ≤ 0.001mg/L and resistant when MICs were >4mg/L; *A. baumanii* isolates were categorized as susceptible when MICs using imipenem were ≤2mg/L and resistant when MICs were >4mg/L).

2.2 Rapid Resalmipinem/Acinetobacter NP test:

The test was performed as previously described (13). *P. aeruginosa* ATCC 27853 was used as a negative control. The resazurin aqueous solution was prepared at a concentration of 0.02%, filtered with a polytetrafluoroethylene filter (0,22 µm pore size). Experiment was performed as follows:

- In columns A, D and G it was added 180µL of Mueller Hinton Broth;
- In columns B, E and H it was added 180µL of Mueller Hinton Broth plus 6.67µg/mL of imipenem to get a final concentration of 6µg/mL;
- 20µL of bacterial suspension (using a 3.0 McFarland standard) was added to each well;
- 20µL of resazurin 0.02% solution were added to each well.

The tray was incubated for 5 hours, with visual inspections every 30 minutes to detect any color change (blue to purple/pink).

3. Results

RESULTS: Overall, 64 clinical isolates were evaluated: 9 *P. aeruginosa* and 55 *A. baumannii*. Table 1, figure 1 present results of *ResaImipinem/Acinetobacter* NP test for both *P. aeruginosa* and *A. baumannii*. The test presented 100% sensitivity (for known resistant strains). Isolates susceptible to imipenem (defined by the gold-standard BMD method) were considered resistant by the *ResaImipinem/Acinetobacter* NP test. Considering species, the test for *A. baumannii* presented 100% sensitivity and specificity. On the other hand, although all *P. aeruginosa* resistant to imipenem were correctly characterized, susceptible isolates presented false positive results.

Table 1: Results of *ResaImipinem/Acinetobacter* NP among clinical isolates of *P. aeruginosa* and *A. baumanni*

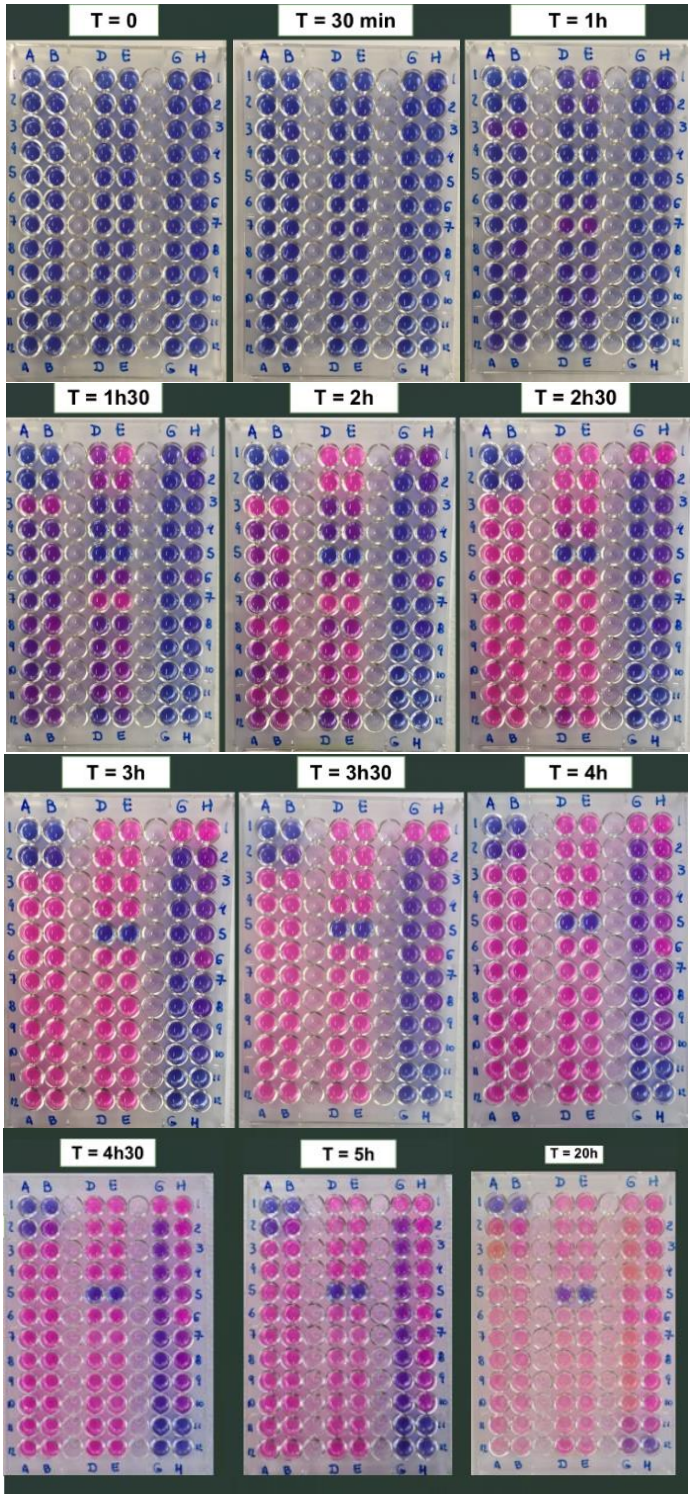
IDENTIFICATION			BMD IMIPENEM		Resalmipenem/ *Acinetobacter* NP test	
#NF	ID	Sampled Material	INTERPRETATION	MIC	Time	Result
1	*P. aeruginosa*	Tracheal Aspirate	R	64	5h00	Positive
2	*P. aeruginosa*	Urine	R	64	5h00	Positive
3	*P. aeruginosa*	Tracheal Aspirate	R	>256	5h00	Positive
4	*P. aeruginosa*	Urine	R	16	5h00	Positive
6	*P. aeruginosa*	LBA	S	1	5h00	Positive
7	*P. aeruginosa*	Urine	R	32	5h00	Positive
8	*P. aeruginosa*	Urine	R	8	5h00	Positive
9	*P. aeruginosa*	Tracheal Aspirate	I	4	4h00	Positive
10	*P. aeruginosa*	Tracheal Aspirate	S	1	5h00	Positive
278	*A. baumannii*	Sputum	R	8	2h00	Positive
279	*A. baumannii*	Sputum	R	8	3h00	Positive
281	*A. baumannii*	Tracheal Aspirate	R	16	2h30	Positive
282	*A. baumannii*	Soft Tissue	R	8	2h30	Positive
283	*A. baumannii*	Sputum	R	8	3h00	Positive
284	*A. baumannii*	Abscess	R	8	2h30	Positive
285	*A. baumannii*	Abscess	R	8	2h00	Positive
286	*A. baumannii*	Tracheal Aspirate	R	32	3h00	Positive

Table 1 (continuation)

289	*A. baumannii*	Tracheal Aspirate	R	32	2h00	Positive
290	*A. baumannii*	Tracheal Aspirate	R	32	3h00	Positive
291	*A. baumannii*	Hemoculture	R	8	2h00	Positive
293	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
294	*A. baumannii*	Burn Wound	R	8	2h00	Positive
296	*A. baumannii*	Tracheal Aspirate	R	32	2h00	Positive
298	*A. baumannii*	Sputum	R	16	2h00	Positive
300	*A. baumannii*	Tracheal Aspirate	R	32	2h30	Positive
301	*A. baumannii*	Tracheal Aspirate	R	32	3h00	Positive
304	*A. baumannii*	Tracheal Aspirate	R	8	2h30	Positive
306	*A. baumannii*	Sputum	R	16	2h30	Positive
308	*A. baumannii*	Tracheal Aspirate	R	64	2h30	Positive
309	*A. baumannii*	Hemoculture	R	32	2h30	Positive
310	*A. baumannii*	Sputum	R	16	2h30	Positive
314	*A. baumannii*	Hemoculture	R	16	2h00	Positive
315	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
316	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
317	*A. baumannii*	Hemoculture	R	32	2h00	Positive
318	*A. baumannii*	Tracheal Aspirate	R	8	2h00	Positive
319	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
320	*A. baumannii*	Tracheal Aspirate	R	8	2h30	Positive
321	*A. baumannii*	Hemoculture	R	64	2h30	Positive
322	*A. baumannii*	Biological Fluid	R	16	2h30	Positive
325	*A. baumannii*	Tracheal Aspirate	R	32	3h00	Positive
326	*A. baumannii*	Hemoculture	R	16	2h00	Positive
330	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
331	*A. baumannii*	Tracheal Aspirate	R	64	1h30	Positive
332	*A. baumannii*	Sputum	R	8	2h00	Positive
337	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
342	*A. baumannii*	Excretion	R	8	2h00	Positive
343	*A. baumannii*	Burn wound	R	16	2h00	Positive
344	*A. baumannii*	Tracheal Aspirate	R	32	2h00	Positive
345	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
383	*A. baumannii*	Tracheal Aspirate	R	32	2h00	Positive
384	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
385	*A. baumannii*	Urine	R	16	2h00	Positive
386	*A. baumannii*	Hemoculture	R	16	1h30	Positive
387	*A. baumannii*	Tracheal Aspirate	R	8	1h30	Positive
394	*A. baumannii*	Sputum	R	32	2h30	Positive
395	*A. baumannii*	Tracheal Aspirate	R	64	2h30	Positive
397	*A. baumannii*	Tracheal Aspirate	R	32	2h00	Positive
398	*A. baumannii*	Sputum	R	32	1h30	Positive
404	*A. baumannii*	Urine	R	32	2h00	Positive
405	*A. baumannii*	Tracheal Aspirate	R	16	2h00	Positive
406	*A. baumannii*	Tracheal Aspirate	R	32	2h00	Positive
407	*A. baumannii*	Tracheal Aspirate	R	32	2h30	Positive
408	*A. baumannii*	Burn wound	R	32	2h30	Positive

Fig. 1: Results of *Resalmipinem/Acinetobacter* NP test from t=0 to 5h00 (with a 30 minute interval check) and then, 20h00 after the incubation.

Description:
without imipenem/cilastatin: A, D, G columns
with imipenem/cilastatin: B, E, H columns
Sterile controls: GH12: imipenem-cilastatin only
AB1 DE5: saline only
GH11: MHB2 only
Pseudomonas spp.: AB2 (negative control) & GH 2-10 (tested samples)
A. baumannii: remaining wells



4. Discussion: Because of the emergence and spread of carbapenem resistance among gram-negative bacilli, the need for rapid methodology to detect this resistance has become even more important (13). Resazurin is an atoxic purple phenoxazine dye, weakly fluorescent that is converted to resorufin (pink) in the presence of amines present in cellular oxidoreductases , that causes the deoxygenation of the N-oxide group and drags down the pH below turning point 6.5. The concentration of the suspension containing resazurin directly determines the time-point for a visible irreversible conversion from blue (above pH 6.5) to a highly fluorescent pink (pH 6.5 to 3.8), noting that when the medium becomes more acidic than pH 3.8 the dye is reversibly reduced to the colorless dihydroresorufin by deoxygenation and further reduction by organic compounds produced through bacterial metabolism, which was observed 20 hours after the tray incubation (14,15,16).

In this experience resazurin was used as the indicator, using a 6µg/mL concentration of imipenem-cilastatin, a value that is subtly above that defines imipenem resistance. (17).

Resistance to carbapenems among these non-fermenting bacteria are endemic in Brazil and, in our setting, isolates presenting susceptibility to carbapenem has become a minority group (18,19), therefore no sensitive *A. baumannii* strain was found when the tests were being conducted. Therefore, new combinations of β-lactams and β-lactamase inhibitors are being used to treat infections caused by carbapenem-resistant strains (20).

The test showed rapid (around 2h30 after tray incubation) and well defined results for all of the *A. baumannii* isolates, whilst not the same was observed on *P. aeruginosa* isolates, which required approximately 5 hours to start showing results, including a high rate of false positives, as described on Table 1, where the turning point to positive was similar for both resistant and sensitive strains (5h00). This happened probably due to the prolonged incubation time necessary to offer any result. Reducing the broth pH slightly was a condition tested in an attempt to improve to reduce the incubation time to get a turning point for *P. aeruginosa* but it didn't help to promote better results. *P. aeruginosa* expresses cytochrome c oxidase (oxidase positive), while *A. baumannii* doesn't (oxidase negative). This might be a key metabolism difference between the species that affects the applicability of the *ResaImipinem /Acinetobacter* NP test for *P. aeruginosa*. Different types of cytochrome c are expressed under different conditions, such as oxygen level and nutrient starvation, others are constitutive (21).

5. *Conclusion:* The *ResaImipinem /Acinetobacter* NP test seemed to be viable, rapid and accurate for *Acinetobacter baumannii* but not for *P. aeruginosa*. Further conditions and different dyes can be tested in order to obtain optimal results for *P. aeruginosa* as well. We recommend to use a larger number of samples tested.

Ethical approval

This article does not contain any studies with human participants or animals performed by any of the authors.

Declaration of Competing Interest

The authors declare that they have no conflict of interest.

Data availability

No data was used for the research described in the article.

References:

1 Strateva, T., & Yordanov, D. (2009). *Pseudomonas aeruginosa* - a phenomenon of bacterial resistance. Journal of medical microbiology, 58(Pt 9), 1133–1148. https://doi.org/10.1099/jmm.0.009142-0

2 Howard, A., O'Donoghue, M., Feeney, A., & Sleator, R. D. (2012). *Acinetobacter baumannii*: an emerging opportunistic pathogen. Virulence, 3(3), 243-250

3 Qin, S., Xiao, W., Zhou, C., Pu, Q., Deng, X., Lan, L., Liang, H., Song, X., & Wu, M. (2022). *Pseudomonas aeruginosa*: pathogenesis, virulence factors, antibiotic resistance, interaction with host, technology advances and emerging therapeutics. Signal transduction and targeted therapy, 7(1), 199. https://doi.org/10.1038/s41392-022-01056-1

4 Jurado-Martín, I., Sainz-Mejías, M., & McClean, S. (2021). *Pseudomonas aeruginosa*: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors. International journal of molecular sciences, 22(6), 3128. https://doi.org/10.3390/ijms22063128

5 Meletis, G., Exindari, M., Vavatsi, N., Sofianou, D., & Diza, E. (2012). Mechanisms responsible for the emergence of carbapenem resistance in *Pseudomonas aeruginosa*. Hippokratia, 16(4), 303–307

6 World Health Organization. WHO publishes list of bacteria for which new antibiotics are urgently needed. 27 feb 2017. Available from: <https://www.who.int/news/item/27-02-2017-who-publishes-list-of-bacteria-for-which-new-antibiotics-are-urgently-needed>

7 Lambert P. A. (2002). Mechanisms of antibiotic resistance in *Pseudomonas aeruginosa*. Journal of the Royal Society of Medicine, 95 Suppl 41(Suppl 41), 22–26.

8 Onguru, P., Erbay, A., Bodur, H., Baran, G., Akinci, E., Balaban, N., & Cevik, M. A. (2008). Imipenem-resistant *Pseudomonas aeruginosa*: risk factors for nosocomial infections. Journal of Korean medical science, 23(6), 982–987. https://doi.org/10.3346/jkms.2008.23.6.982

9 Buehrle, D. J., Shields, R. K., Clarke, L. G., Potoski, B. A., Clancy, C. J., & Nguyen, M. H. (2016). Carbapenem-Resistant *Pseudomonas aeruginosa* Bacteremia: Risk Factors for Mortality and Microbiologic Treatment Failure. Antimicrobial agents and chemotherapy, 61(1), e01243-16. https://doi.org/10.1128/AAC.01243-16

10 Colquhoun, J. M., Farokhyfar, M., Hutcheson, A. R., Anderson, A., Bethel, C. R., Bonomo, R. A., Clarke, A. J., & Rather, P. N. (2021). OXA-23 β-Lactamase Overexpression in Acinetobacter baumannii Drives Physiological Changes Resulting in New Genetic Vulnerabilities. mBio, 12(6), e0313721. https://doi.org/10.1128/mBio.03137-21

11 Evans, B. A., & Amyes, S. G. (2014). OXA β-lactamases. Clinical microbiology reviews, 27(2), 241–263. https://doi.org/10.1128/CMR.00117-13

12 Ferrer, R., Martin-Loeches, I., Phillips, G., Osborn, T. M., Townsend, S., Dellinger, R. P., ... & Levy, M. M. (2014). Empiric antibiotic treatment reduces mortality in severe sepsis and septic shock from the first hour: results from a guideline-based performance improvement program. Critical care medicine, 42(8), 1749-1755

13 Nordmann, P., Sadek, M., Tinguely, C., & Poirel, L. (2021). Rapid ResaImipenem/Acinetobacter NP test for detection of carbapenem susceptibility/resistance in *Acinetobacter baumannii*. Journal of Clinical Microbiology, 59(6), e03025-20

15 Hudman, D. A., & Sargentini, N. J. (2013). Resazurin-based assay for screening bacteria for radiation sensitivity. SpringerPlus, 2(1), 1-6

16 Dai, J., Tang, H., Li, X., Santini, C. L., Cui, W., Liu, N., ... & Wu, L. F. (2021). Resazurin as an indicator of reducing capacity for analyzing the physiologic status of deep-sea bacterium *Photobacterium phosphoreum* ANT-2200. Journal of Oceanology and Limnology, 39(1), 297-305

17 Zhanel, G. G., Simor, A. E., Vercaigne, L., Mandell, L., & Canadian Carbapenem Discussion Group (1998). Imipenem and meropenem: Comparison of in vitro activity, pharmacokinetics, clinical trials and adverse effects. The Canadian journal of infectious diseases = Journal canadien des maladies infectieuses, 9(4), 215–228. https://doi.org/10.1155/1998/831425

18 Hilmar Wisplinghoff et al. (2017) 181 - Pseudomonas spp., Acinetobacter spp. and Miscellaneous Gram-Negative Bacilli. Infectious Diseases (Fourth Edition) Volume 2, 2017, Pages 1579-1599.e2

19 Fonseca, E. L., Scheidegger, E., Freitas, F. S., Cipriano, R., & Vicente, A. C. P. (2013). Carbapenem-resistant *Acinetobacter baumannii* from Brazil: role of carO alleles expression and blaOXA-23 gene. BMC Microbiology, 13(1). http://dx.doi.org/10.1186/1471-2180-13-245

20 Moreira, N. K., & Caierão, J. (2021). Ceftazidime-avibactam: are we safe from class A carbapenemase producers' infections?. Folia microbiologica, 66(6), 879–896. https://doi.org/10.1007/s12223-021-00918-5

21 Arai H. (2011). Regulation and Function of Versatile Aerobic and Anaerobic Respiratory Metabolism in Pseudomonas aeruginosa. Frontiers in microbiology, 2, 103. https://doi.org/10.3389/fmicb.2011.00103

ANEXOS

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