


The impact of efflux pumps on meropenem susceptibility among metallo- β -lactamase-producing and nonproducing *Pseudomonas aeruginosa*: Insights for better antimicrobial stewardship

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To the Editor—In the past several decades, carbapenemases have emerged as the main carbapenem-resistance mechanism in *Pseudomonas aeruginosa*, *Acinetobacter* spp, and more recently, among Enterobacteriales.¹ In Brazilian hospitals, *bla*_{SPM-1} and *bla*_{IMP-1} have been described as the most prevalent metallo- β -lactamase (MBL) genes among *P. aeruginosa* isolates.² However, efflux pumps may contribute to carbapenem resistance development in *P. aeruginosa* because accumulation of the drug is reduced due to its rapid extrusion from the bacterial cell.³ Considering the lack of activity of carbapenem agents in front of these mechanisms, drugs such as ceftolozane/tazobactam (CT), have recently been targeted for new therapeutic approaches. Thus, it is crucially important to determine the exact mechanism that is occurring to establish a better stewardship strategy because CT does not have action on MBL-producing *P. aeruginosa*.⁴

In *P. aeruginosa*, efflux pumps have been categorized into 5 superfamilies, 4 of which belong to the resistance-nodulation division (RND) family: MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY.⁵ Efflux pump overexpression, particularly MexAB-OprM and MexXY, may cause high levels of carbapenem resistance among *P. aeruginosa* isolates.⁶ Although carbapenemases and efflux pumps have an important role in determining resistance, it is particularly difficult to infer the exact influence of each other because multiple resistance mechanisms may occur simultaneously.

The aim of this study was to evaluate the meropenem minimum inhibitory concentration (MIC) among meropenem-nonsusceptible *P. aeruginosa*, producing or non-producing MBLs, in the presence of a specific pump inhibitor agent, the L-phenyl-L-arginine β -naphthylamide (Pa β N).⁷ Moreover, ceftolozane/tazobactam (C/T) MICs were determined for all isolates included in this study.

A set of 91 meropenem-nonsusceptible *P. aeruginosa* were consecutively included in this study. Only 1 isolate per patient was considered, which had been recovered from distinct clinical specimens (endotracheal aspirate, n = 62; sputum, n = 10; urine, n = 10; and others, n = 9) from hospitalized patients in southern Brazil from August 1, 2016, through February 2, 2017.

Meropenem MICs were determined by standard broth microdilution, according to the Clinical Laboratory Standards Institute (CLSI) protocol using *P. aeruginosa* ATCC 27853 and *Escherichia*


coli ATCC 25922 as strains for quality control.⁸ C/T MICs were determined by Etest (Liofilchem, Italy). For MBL gene detection, a real-time polymerase chain reaction (PCR) was performed as previously described.² Broth microdilution using meropenem as substrate, ranging from 0.5 to 256 μ g/mL, in the presence of 50 μ g/mL Pa β N, was performed to determine the MexAB-OprM overexpression activity, as described elsewhere.⁷ Efflux overexpression was determined when a decrease in meropenem MIC without inhibitor occurred at $\geq 2\log_2$.⁹ Isolates were evaluated in triplicate, and the results were visually screened after 24 hours of incubation at 35°C.

Among our set of isolates, 15 (16.5%) were MBL producers (13 *bla*_{SPM-1} and 2 *bla*_{IMP}), and among them, high levels of meropenem resistance (MICs ≥ 128.0 μ g/mL) were found. The remaining 76 (83.5%) isolates were non-MBL producers with meropenem MICs ranging from 8.0 to 256.0 μ g/mL (Table).

In the presence of Pa β N, all *P. aeruginosa* isolates showed a significant reduction in meropenem MICs, no matter the presence of any MBL gene (Table 1). Moreover, overexpression of the MexAB-OprM efflux pump was verified for all meropenem-resistant non-MBL-producing isolates. This result is in agreement with other studies, which have reported that >70% of the isolates can overexpress efflux pump mechanisms like MexAB-OprM.³

This study highlights important issues: (1) the need to detect the exact carbapenem resistance mechanism involved (a simple phenotypic test such as blue-carba would be enough) because MBL producers are not elective for CT therapy; (2) better targeting of the use of new antimicrobials, such as CT, for those resistant isolates due to the overexpression of ampC enzyme associated with efflux pumps (ie, CT emerged as an important therapeutic agent with no resistance detected in this study and with low MIC₅₀ and MIC₉₀ of 1.0 and 2.0 μ g/mL, respectively [data not shown]); and (3) epidemiology survey of *Pseudomonas* resistance (currently, carbapenem-resistance due to efflux plus porin loss represent nearly 70% of all carbapenem-resistant *Pseudomonas* isolates) for rapid adjustment in stewardship programs to avoid antimicrobial resistance.

In conclusion, the presence of an efflux pump inhibitor (Pa β N) markedly decreases the level of meropenem resistance among *P. aeruginosa* isolates,¹⁰ regardless the presence of MBL, highlighting the role of efflux pump as an important mechanism to keep a high level of meropenem resistance. Additionally, microbiology labs should detect and report the presence of the resistance mechanism to establish the best antimicrobial use, particularly to prevent antimicrobial resistance development.

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Table 1. Effect of the Efflux Pump Inhibitor on Ceftolozane/Tazobactam and Meropenem MIC Values Among Meropenem-Nonsusceptible MBL-producing and Non-MBL-producing *Pseudomonas aeruginosa*

No. of Isolates	Efflux Pump	MBL Gene	C/T MIC ^a Range, µg/mL	Meropenem MIC ^b Range, µg/mL	
				Without PaβN	With PaβN
2	MexAB-XY	<i>bla</i> _{IMP}	>256.0	256.0–128.0	2.0–8.0
12	MexAB-XY	<i>bla</i> _{SPM-1}	>256.0	256.0	4.0–8.0
1	MexAB	<i>bla</i> _{SPM-1}	>256.0	256.0	16.0
10	MexXY	None	0.75–4.0	256.0	8.0–16.0
8	MexXY	None	1.0–2.0	8.0–64.0	1.0
6	MexXY	None	1.0	16.0	0.5–1.0
16	MexXY	None	1.0–2.0	8.0–32.0	0.5–1.0
12	MexAB	None	0.75–2.0	16.0–32.0	1.0
10	MexAB	None	1.0	16.0	1.0–2.0
14	MexAB	None	1.0–4.0	8.0–32.0	1.0–2.0

Note. MBL, metallo-β-lactamase; MIC, minimum inhibitory concentration; PaβN, L-phenyl-L-arginine β-naphthylamide.

^aC/T (ceftolozane/tazobactam) break points are ≤4 µg/mL (susceptible) and >4 µg/mL (resistant).

^bMeropenem break points are ≤4 µg/mL (susceptible) and ≥16 µg/mL (resistant).


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Forecasting from phenotypic testing to an antimicrobial stewardship strategy: Does the time to positivity of a blue-carba test predict the meropenem susceptibility level among carbapenemase producers?

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To the Editor—Currently, carbapenem-resistant *Enterobacterales* (CRE) have emerged worldwide at an alarming rate, causing both community and nosocomial infections.¹ Carbapenem-resistance may be related to a decrease in bacterial outer-membrane