

**In-vitro evaluation of a dual carbapenem combination
against carbapenemase-producing *Acinetobacter
baumannii***

Sir,

Russo and colleagues, in this Journal, recently reported a multi-center study of bloodstream infections caused by carbapenem-resistant *A. baumannii* (CRAB).¹ This underlines that the spread of CRAB is becoming a major public health concern, being listed among the top three priorities for multidrug-resistant bacteria of the World Health Organization.^{2,3} It is known that almost all carbapenem-resistant *A. baumannii* produce a carbapenemase.³ The types of carbapenemases encountered are mostly specific to the *Acinetobacter* genus.^{2,3} These are mainly Ambler class D β -lactamases possessing carbapenemase activity such as the overexpressed and naturally-occurring OXA-51 and the acquired enzymes of the OXA-23-, OXA-40- and OXA-58-types as well as the rare OXA-143.³ The OXA-23-type enzymes are the most widespread carbapenemases in *A. baumannii*.³ In addition, few other carbapenemases have been identified in *A. baumannii*, corresponding to the Ambler class A β -lactamases of the KPC-type, and the Ambler class B metallo- β -lactamases of the IMP, VIM and NDM types.³ The CRAB clinical isolates are in their vast majority resistant to many other important antibiotics such as all β -lactams, fluoroquinolones and aminoglycosides, making related infections challenging to treat. As opposed to carbapenem-resistant Enterobacterales, the prevalence of CRAB among *Acinetobacter* sp is quite high, ranging from 40 to 80%, worldwide.⁴

None of the novel β -lactam/ β -lactamase inhibitor combinations currently available (ceftazidime/avibactam, ceftolozane/tazobactam, imipenem/relebactam, meropenem/ varbobactam) are efficient for treating infections due to CRAB since none of those antibiotics is active against class D carbapenemase producers. Therefore, very few therapeutic options are available currently for treating MDR *A. baumannii*,^{1,5} and most of them include antibiotic combinations with polymyxins or tigecycline which efficacy remains debatable.¹ In addition, polymyxins and tigecycline molecules raise concern of poor diffusion and possible toxicity.

Early studies performed using animal models of infections or experimental treatments of humans indicate that dual-carbapenem combinations may be efficient for treating infections due to KPC and NDM producing Enterobacterales, and particularly *Klebsiella pneumoniae*.⁶⁻¹⁰ Further in-vitro and in-vivo studies validated those findings.⁶⁻⁹ The rationale of using two carbapenems (in particular ertapenem with another carbapenem) would be that ertapenem may bind to the active site of the carbapenemase with high affinity, and therefore may prevent the hydrolysis of the other carbapenem molecule. The mode of action of these dual

Table 1
Evaluation of the efficacy of a dual carbapenem combination against carbapenemase-producing *Acinetobacter baumannii*.

Strain	Country of isolation	Carbapenemases	MIC (mg/L)		FIC
			IPM	MEM	
R629	France	OXA-23	64	128	0.50
R625	France	OXA-23	32	64	0.50
R628	France	OXA-23	32	32	0.75
R627	Bahrein	OXA-23	32	32	1
R622	France	OXA-23	64	64	0.75
R626	Bahrein	OXA-23	32	64	0.75
R623	Saudi Arabia	OXA-23	32	64	0.50
R624	Colombia	OXA-23	8	64	1
N715	France	OXA-23	16	16	0.75
N688	Switzerland	OXA-23	32	32	1
N690	Switzerland	OXA-23	64	64	0.50
R703	Brazil	OXA-40	64	64	0.60
R704	France	OXA-40	64	64	0.60
R824	Turkey	OXA-58	32	64	0.50
R825	Romania	OXA-58	32	8	0.75
N7	Switzerland	OXA-72	64	128	0.75
N420	Switzerland	NDM-1	8	8	0.60
N15	Switzerland	NDM-1	32	32	0.75
R32	France	NDM-1	32	32	0.60
R34	France	NDM-1	16	64	0.50
N655	Switzerland	NDM-1	64	64	1
N739	Switzerland	NDM-5	128	64	0.60
R71	France	IMP-4	16	32	0.75

IPM: Imipenem; MEM: Meropenem.

combinations would be somehow similar to that of amoxicillin or ceftazidime combined with the β -lactamase inhibitors clavulanic acid or avibactam, respectively.

To the best of our knowledge, dual carbapenem combinations have never been tested against carbapenemase-producing *A. baumannii*. Therefore, our goal was to evaluate the in-vitro activity of a dual carbapenem combination, i.e. imipenem and meropenem, against *A. baumannii* isolates producing different types of carbapenemases. Imipenem and meropenem were retained since those carbapenems are widely used for treating infections caused by *A. baumannii*. Ertapenem was not tested since *A. baumannii* is naturally resistant to ertapenem, likely related to its weak penetration through the outer membrane.

A collection of twenty three non-clonally related *A. baumannii* isolates recovered from clinical samples from Barhein, Brazil, Colombia, France, Saudi Arabia, Switzerland, and Turkey was tested. All the isolates were non susceptible to imipenem and meropenem (Table 1). Following the latest EUCAST breakpoints (<http://www.eucast.org/>), isolates with MIC values of imipenem and meropenem ≤ 2 mg/L are categorized as susceptible, whereas those with MICs of imipenem >4 mg/L or and MIC of

meropenem >8 mg/L are considered resistant. The nature of the carbapenemase produced by each isolate was characterized at the molecular level. Our collection included eleven OXA-23, two OXA-40, two OXA-58, one OXA 72, six NDM and one IMP producers that mirrors the variety of acquired carbapenemases identified in *A. baumannii* and their distribution worldwide (Table 1). Fractional inhibitory concentration (FICs) index were calculated according to the following formula, $\Sigma FIC = FIC \text{ of imipenem} + FIC \text{ of meropenem}$ where $FIC \text{ of imipenem or meropenem} = MIC \text{ of imipenem or meropenem in combination, divided by the MIC of imipenem or meropenem alone}$. Interpretation of the results was based on the following; FIC values of ≤ 0.5 indicate synergy, FIC values of >0.5 to 4 indicate no interaction, and FIC values >4 indicate antagonism.¹¹

Six out of the twenty three isolates tested showed synergy activity of imipenem and meropenem (ca. 25%) whereas the combination of imipenem and meropenem were indifferent for the other tested carbapenemase-producing isolates. Among the six isolates for which a synergy was identified, different types of carbapenemases were produced. No antagonism between imipenem and meropenem was identified for any of the isolates.

The rate of in-vitro synergy observed between imipenem and meropenem for those CRAb corresponds to that identified for carbapenemase-producing *K. pneumoniae*.¹¹ In addition, we identified here a synergy for a single *A. baumannii* isolate producing NDM-1 whereas that synergy as determined by the results of the FIC values was not identified for any of the NDM-1-producing *K. pneumoniae* tested previously.¹¹

Our study suggests that dual carbapenem combination may be effective, to some extent, against some carbapenemase-producing *A. baumannii*. This dual combination may therefore work in some cases to inhibit not only the activity of carbapenemases of class A (KPC) and class B (NDM) as known in Enterobacteriales, but also of those peculiar class D carbapenemases specific to *A. baumannii* (OXA-23, OXA-40 and OXA-58). Due to the dearth of novel antibiotics for treating infections due to MDR *A. baumannii*, this dual carbapenem combination may offer an alternative since all MDR *A. baumannii* produce a carbapenemase. By targeting the inactivation of the carbapenemase, we are targeting actually the MDR pattern of those strains.

Further investigations using animal models of infection and clinical trials are required to further establish this treatment as an alternative against MDR *A. baumannii*.

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Declaration of Competing Interest

None to declare.

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