Co-occurrence of carbapenems in *Acinetobacter baumannii*, a dream or reality?

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Introduction

Acinetobacter baumannii is an important opportunistic pathogen that is rapidly evolving towards multidrug resistance and is responsible for life-threatening infections. Carbapenems are commonly used to treat *A. baumannii* infections but emergence of carbapenemase encoding genes, such as $bla_{\text{OXA23-like}}$, $bla_{\text{OXA24-like,}}$ $bla_{\text{OXA58-like,}}$ and bla_{NDM} has been recently reported. Moreover, several studies have reported the co-occurence of two distinct carbapenemases in some isolates. The aim of the present study was to study such isolates to demonstrate that this phenomenon was in fact due to the existence of different bacterial clones harboring different genes.

Materials

We studied 6 strains of *A. baumannii*: 1 containing $bla_{\text{OXA23-like}}$ and $bla_{\text{OXA24-like}}$ genes and 5 with $bla_{\text{OXA23-like}}$ and bla_{NDM} genes.

Strain	Hospital location	Ward	Date of isolation (mo/yr)	Sample	Carbapenemase genes	ST type
AH35	Sétif	Intensive Care Unit	05/2011	Urine	Oxa23-Oxa24	2
924	Algiers	Pus unit	04/2011	Pus	Oxa23-NDM-1	2
519	Algiers	Intensive Care Unit	03/2013	NA	Oxa23-NDM-1	25
598	Algiers	Burns	03/2013	NA	Oxa23-NDM-1	25
624	Algiers	Intensive Care Unit	03/2013	NA	Oxa23-NDM-1	25
679	Algiers	Pediatric	03/2013	NA	Oxa23-NDM-1	25

NA: Not available

References

(1) Kempf M, et al. Emergence of resistance to carbapenems in *Acinetobacter baumannii* in Europe: clinical impact and therapeutic options. Int J Antimicrob Agents 2012 Feb;39(2):105-14.

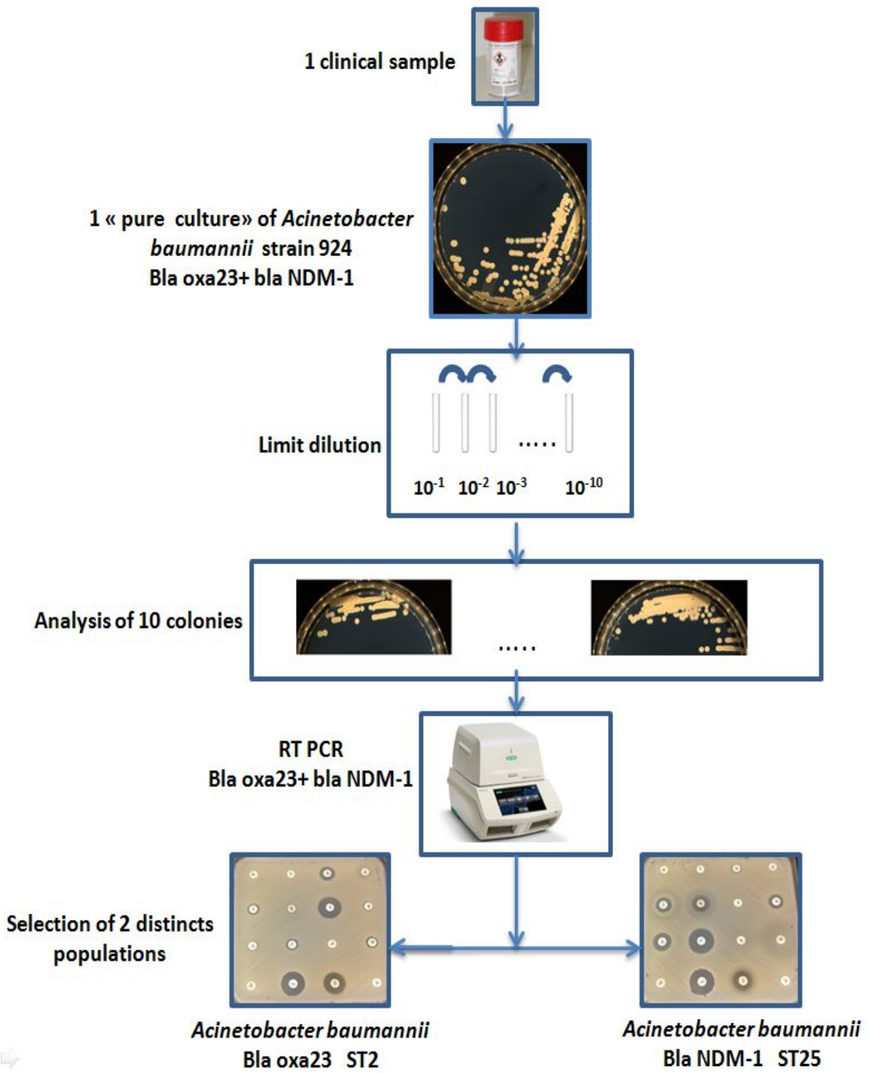
(2) Bakour S, et al. First report of 16S rRNA methylase ArmA-producing *Acinetobacter baumannii* and rapid spread of metallo-beta-lactamase NDM-1 in Algerian hospitals. J Infect Chemother 2014 Nov;20(11):696-701.

(3) Bakour S, et al. Carbapenemase-producing *Acinetobacter baumannii* in two university hospitals in Algeria. J Med Microbiol 2012 Sep;61(Pt 9):1341-3.

(4) Zhou S, et al. "Roar" and blaNDM-1 and "silence" of blaOXA-58 co-exist in *Acinetobacter pittii.* Sci Rep 2015;5:8976.

Methods

Each strain was subcultured 10 fold in limit dilution in water. Every dilution was cultivated on Trypticase Soy agar plates for 24h at 37°C and isolated bacteria was analysed. Antibacterial susceptibility testing, real time PCR for detection of antibiotic resistance genes and multilocus sequence typing were performed on strains before and after limit dilution.

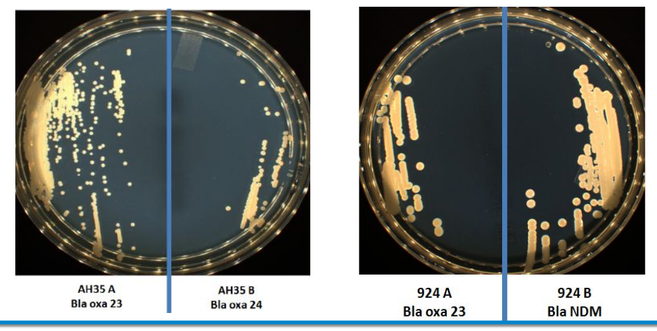


Results

For each strain with co-occurence of carbapenemases, 10 colonies were selected after limit dilutions so a total of 100 clones were studied. For example, from the strain AH35 containing $bla_{\text{OXA23-like}}$, and $bla_{\text{OXA24-like,}}$ we isolated after limit dilution 6 colonies containing only $bla_{\text{OXA23-like}}$ and 4 with only $bla_{\text{OXA24-like..}}$ Each population has the same ST type (ST 2) but different antibiotic susceptibility testing. For the strain 924 containing $bla_{\text{OXA23-like}}$, and $bla_{\text{NDM,}}$ we differentiate 2 populations with different carbapenemase encoding genes, different resistance phenotypes and also different STs i.e 5 clones with $bla_{\text{OXA23-like}}$ (ST 2) and 5 clones with bla_{NDM} gene (ST 25). For 2 strains on 6 tested, we separated 2 different populations of *A. baumannii,* each of them with a different carbapenemase, also a different antibiotic susceptibility testing and often different clonal types.

Strain	Carbapenemase genes	ST type	Etest IPM (µg/mL)	CTX	CAZ	TIC	TIM	TPZ	ATM	IPM	CN	TOB	AK	K	CIP	SXT	RA	DO	CT
924	Oxa23-NDM-1	2	>32	R	R	R	R	R	R	R	R	R	R	R	R	R	S	R	S
924-A	Oxa23	2	>32	R	R	R	R	R	I	R	S	S	R	R	R	R	S	R	S
924-B	NDM-1	25	>32	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S
AH35	Oxa23-Oxa72	2	>32	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S
AH35-A	Oxa23	2	>32	R	R	R	R	R	R	R	S	S	R	S	R	R	S	R	S
AH35-B	Oxa72	2	>32	R	R	R	R	R	R	R	R	R	R	S	R	R	S	R	S

Moreover the 2 different populations had a different aspect on agar plate.



Conclusions

Here we report that the coexistence of two carbapenemases in single isolates of *A. baumannii* is likely due to the existence of different clones harboring different carbapenemases encoding genes. This result is the first to our knowledge to demonstrate that *A. baumannii* infections could be linked to multiple clones.