Rev. Inst. Med. trop. S. Paulo
51(2):111-113, March-April, 2009
doi: 10.1590/S0036-46652009000200010

CASE REPORT

INDICATIONS OF CARBAPENEM RESISTANCE EVOLUTION THROUGH HETERORESISTANCE AS AN INTERMEDIATE STAGE IN *Acinetobacter baumannii* AFTER CARBAPENEM ADMINISTRATION

Silvana Vargas SUPERTI(1), Daniela de Souza MARTINS(1), Juliana CAIERÃO(1), Fabiana da Silva SOARES(1), Taísa PROCHNOW(1) & Alexandre Prehn ZAVASCKI(2)

SUMMARY

We describe an *in vivo* evolution of an antimicrobial profile from susceptibility to full-resistance to carbapenems, with heteroresistance as an intermediate stage, in an *Acinetobacter baumannii* strain. Heteroresistance was characterized by the growth of sub-populations within the susceptibility halo in both disk-diffusion and Etest. PCRs for the main *A. baumannii* carbapenemases were negative. The exact resistance mechanism, diagnostic methods and clinical relevance of heteroresistance in *A. baumannii* warrant further investigations. This is the first description of such phenomenon *in vivo* and the second report of heteroresistance to carbapenems in *A. baumannii*.

KEYWORDS: *Acinetobacter baumannii;* Carbapenem-heteroresistance; Meropenem treatment.

INTRODUCTION

Acinetobacter baumannii, which is associated with a wide range of infections, particularly pneumonia among intensive care unit (ICU) patients, has emerged as an important nosocomial pathogen in many parts of the world[4]. The carbapenems, imipenem and meropenem, are among the drugs of choice used to treat nosocomial infections due to multidrug-resistant *A. baumannii* isolates[4].

As other Gram-negative rods, resistance in these bacteria is known to be homogeneous within a culture although a report has described the spread of hetero-carbapenem-resistant *A. baumannii* isolates[7]. In the present report, we describe the recovery from blood of an *A. baumannii* isolate presenting heteroresistance to carbapenems in a patient receiving meropenem due to an intra-abdominal abscess.

CASE REPORT

In March 2006, a first *A. baumannii* resistant to both imipenem and meropenem was identified in our institution[12]. Two isolates were recovered from an intra-abdominal abscess secretion and from the blood of a 67-year-old female patient at ICU, respectively. Identification of the isolates has been based on biochemical tests, including mobility, oxidase activity, oxidation of glucose on OF-medium; and API 20 NE method (6.0 version, bio-Merieux, Marcy L'Etoile, France). This patient was receiving I.V. meropenem (1g IV every eight hours) for exactly 31 days, for the treatment of the abscess after an esophagectomy for a carcinoma of the esophagus. The therapy was changed for polymyxin B (75 mg IV every 12 hours) when the culture results became available (three days after collection), but the patient ultimately died of septic shock seven days after starting new antimicrobial treatment.

The phenotypic profile of this isolate showed heteroresistance to both imipenem and meropenem, as indicated by the growth of sub-populations within the clear zone of inhibition, which demonstrated susceptibility (20 and 19 mm, respectively) in the disk-diffusion test (Fig. 1A), performed according to Clinical Laboratory Standards Institute guidelines[3]. The test was repeated with the sub-populations and the same phenotypic profile was observed, i.e. the growth of resistant sub-populations within the zone of inhibition with a diameter indicating susceptibility. These findings were confirmed using the Etest (AB Biodisk, Solna, Sweden) (Fig. 1B). The same phenotypic phenomenon was observed when the sub-populations were re-tested.

Phenotypic tests for MBL detection using a disk approximation test with imipenem (Oxoid) in the presence of EDTA were negative for both isolates. Additionally, PCR for carbapenemases genes (bla_{IMP}, bla_{VIM}, bla_{SPM}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-51} and bla_{OXA-58} was carried out using specific primers as described elsewhere[1-5], but there was no product of amplification for any primers, except for the $bla_{OXA-51.}$

Forty-three days latter, a carbapenem-resistant *A. baumannii* was

(1) Laboratory of Microbiology, Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.
(2) Dept. Infectology, Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Porto Alegre, RS, Brazil.
Correspondence to: Silvana Vargas Superti. Hospital São Lucas da Pontifícia Universidade Católica do Rio Grande do Sul, Laboratório de Microbiologia, Av. Ipiranga 6690, 90610-000 Porto Alegre, RS, Brasil. Tel./Fax: +55.51.33203145. E-mail: silvanas@ufcspa.edu.br

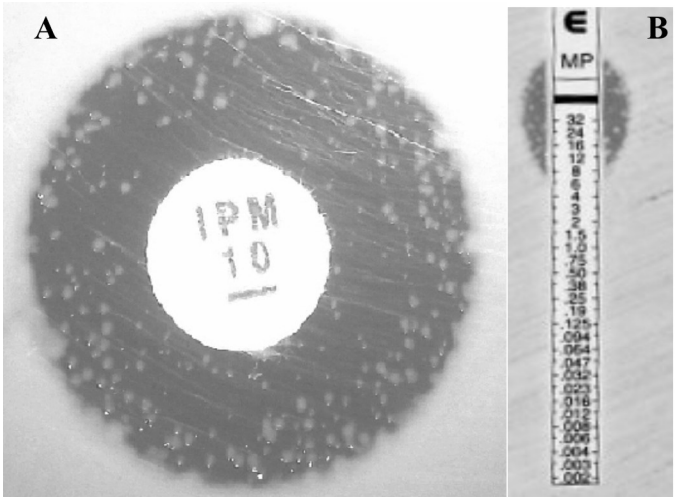

Fig. 1 - Disk-diffusion (A) for imipenem and Etest (B) for meropenem showing the growth of resistant sub-populations of *Acinetobacter baumannii.*

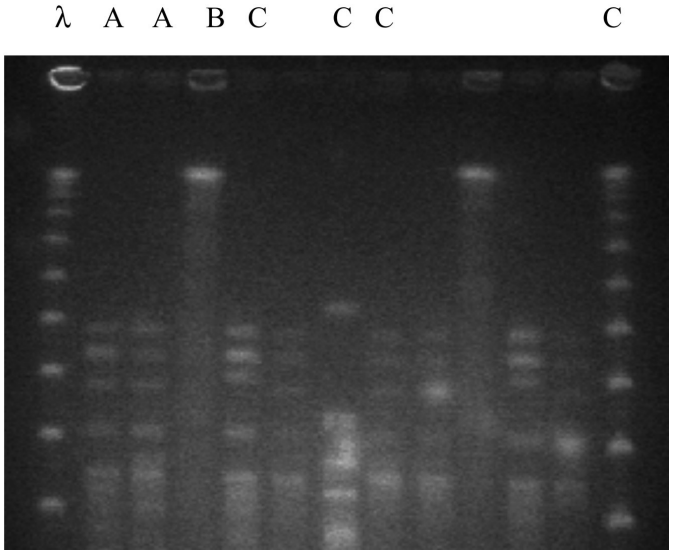

Fig. 2 - Pulsed-field gel electrophoresis showing a high degree of similarity between the heteroresitant isolate (A), fully resistant isolate (B) and carbapenem-susceptible isolates previously recovered in our institution (C).

recovered from a tracheal aspirate of a 44-years male ICU patient, who was under mechanical ventilation due to severe tetanus. This patient received I.V. meropenem (1g t.i.d.) during the previous 12 days as empirical treatment for a ventilator-associated pneumonia. This isolate was fully resistant to imipenem and meropenem (MIC >32 µg/mL for both) by Etest method.

Both hetero and full-resistant isolates were submitted to molecular typing with other *A. baumannii* isolate previously recovered from patients at ICU and proved to be the same strain of a carbapenem-susceptible isolate recovered 102 days before the recovery of the heteroresistant one. This latter patient was a 65-year-old woman with erythematous systemic lupus, who was hospitalized at ICU and presented the *A. baumannii* recovered from the tracheal aspirate and blood. This patient had not received carbapenem.

DISCUSSION

We presented a rare case of a carbapenem-heteroresistant *A. baumannii* recovered from blood and abscess secretion of a patient receiving meropenem therapy. A previous report has described heteroresistance to carbapenems in eight *A. baumannii* isolates which were also neither MBL nor oxacillinase producers[7]. As in this previous study, the exact mechanism of resistance in our strain could not be determined. Although the main *A. baumannii* carbapenemases have been ruled out, the isolate carried a gene encoding an OXA-51-like group β-lactamase, a gene present in most *A. baumannii* strains, but with a non-clearly defined role in carbapenem resistance[11].

Heteroresistance, which may be defined as a phenotypic manifestation of resistance within a genetically homogeneous strain[10], has only rarely been described in Gram-negative bacteria[6-9]. Although authors are not certain about the clinical impact of this resistance phenotype in Gram-negative rods, our report suggests that heteroresistance may adversely affect the outcome of patients, since the *A. baumannii* isolate was recovered from the blood of a patient receiving meropenem therapy.

In conclusion, heteroresistance in nonfermentative Gram-negative bacteria is a rare and poorly understood phenomenon, the exact mechanism determining this phenotype, the best diagnostic methods and its clinical relevance warrant further investigations.

ACKNOWLEDGMENTS

We are very grateful to Líbera Dalla Costa, who had kindly performed PCR tests.

RESUMO

Indicações da evolução da resistência ao carbapenem através da heteroresistência como estágio intermediário no *Acinetobacter baumannii* após administração de carbapenem

Descrevemos a evolução *in vivo*, de um perfil de sensibilidade aos antimicrobianos, passando de sensibilidade a resistência total aos antibióticos carbapenêmicos, com um estágio intermediário de heteroresistência em isolado de *Acinetobacter baumannii*. A heteroresistência foi caracterizada pelo crescimento de sub-população na zona de inibição pelo método de disco-difusão e pelo Etest. PCRs para as principais carbapenemases envolvidas com resistência neste microrganismo foram negativas. O exato mecanismo de resistência envolvido, método diagnóstico e relevância clínica justificam investigação adicional. Esta é a primeira descrição deste fenômeno *in vivo* e o segundo relato de heteroresistência em *A. baumannii*.

REFERENCES

1. AFZAL-SHAH, M.; WOODFORD, N. & LIVERMORE, D.M. - Characterization of OXA-25, OXA-26, and OXA-27, molecular class D beta-lactamases associated with carbapenem resistance in clinical isolates of *Acinetobacter baumannii*. **Antimicrob. Agents Chemother., 45:** 583-588, 2001.

2. BOU, G.; OLIVER, A. & MARTINEZ-BELTRAN, J. - OXA-24, a novel class D beta-lactamase with carbapenemase activity in an *Acinetobacter baumannii* clinical strain. **Antimicrob. Agents Chemother., 44:** 1556-1561, 2000.

3. CLINICAL AND LABORATORY STANDARDS INSTITUTE - M100-S16, Performance Standards for Antimicrobial Susceptibility Testing, 16th Informational Supplement. Wayne, CLSI, 2006.

4. DIJKSHOORN, L.; NEMEC, A. & SEIFERT, H. - An increasing threat in hospitals: multidrug-resistant *Acinetobacter baumannii*. **Nat. Rev. Microbiol., 5:** 939-951, 2007.

5. ELLINGTON, M.J.; KISTLER, J.; LIVERMORE, D.M. & WOODFORD, N. - Multiplex PCR for rapid detection of genes encoding acquired metallo-ß-lactamases. **J. Antimicrob. Chemother., 59:** 321-322, 2007.

6. LI, J.; RAYNER, C.R.; NATION, R.L. *et al.* - Heteroresistance to colistin in multidrug-resistant *Acinetobacter baumannii*. **Antimicrob. Agents Chemother., 50:** 2946-2950, 2006.

7. POURNARAS, S.; IKONOMIDIS, A.; MARKOGIANNAKIS, A.; MANIATIS, A.N. & TSAKRIS, A. - Heteroresistance to carbapenems in *Acinetobacter baumannii*. **J. Antimicrob. Chemother., 55:** 1055-1056, 2005.

8. POURNARAS, S.; IKONOMIDIS, A.; MARKOGIANNAKIS, A. *et al.* - Characterization of clinical isolates of *Pseudomonas aeruginosa* heterogeneously resistant to carbapenems. **J. med. Microbiol., 56:** 66-70, 2007.

9. POURNARAS, S.; IKONOMIDIS, A.; NEOU, E. *et al*. - Piperacillin/tazobactam-heteroresistant *Pseudomonas aeruginosa* from urinary infection, successfully treated by piperacillin/tazobactam. **J. Antimicrob. Chemother., 61:** 757-758, 2008.

10. RINDER, H. - Hetero-resistance: an under-recognized confounder in diagnosis and therapy? **J. med. Microbiol., 50:** 1018-1020, 2001.

11. TURTON, J.F.; WOODFORD, N.; GLOVER, J. *et al.* - Identification of *Acinetobacter baumannii* by detection of the blaOXA-51-like carbapenemase gene intrinsic to this species. **J. clin. Microbiol., 44:** 2974-2976, 2006.

12. ZAVASCKI, A.P.; SOARES, F.C.; SUPERTI, S.V. *et al.* - Stable carbapenem susceptibility rates among multidrug-resistant *Acinetobacter* spp. strains in a setting of high prevalence of carbapenem-resistant *Pseudomonas aeruginosa.* **Int. J. Antimicrob. Agents, 30:** 187-189, 2007.

Received: 9 September 2008
Accepted: 5 March 2009