First case of bacteraemia due to *Acinetobacter schindleri* harbouring bla_{NDM-1} in an immunocompromised patient

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Abstract

Clinically significant NDM-1-producing *Acinetobacter schindleri* has not yet been described in the literature. We report the first case of bacteraemia due to an *A. schindleri* strain harbouring bla_{NDM-1} recovered from an immunocompromised patient. Our report reinforces the fact that NDM-1 can easily be acquired by *Acinetobacter* species.
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Acinetobacter species are opportunistic pathogens that are responsible for nosocomial infections and outbreaks, especially in intensive care units. Most of the clinically significant isolates are resistant to a variety of antibiotics including carbapenems, which are the drug of choice to treat *Acinetobacter* infections.

In past years bla_{NDM-1} has increasingly been spread among Gram-negative bacteria and has been recognized as an important mechanism of carbapenem resistance in *Acinetobacter baumannii* and other species of this genus [1,2]. Several reports of *Acinetobacter* spp. harbouring bla_{NDM} have suggested that *Acinetobacter* could serve as a source and spread of this threatening carbapenemase.

Among the more frequent non-*baumannii Acinetobacter* species causing infections, we found *A. junii, A. soli, A. ursingii, A. nosocomialis* and *A. lwoffii* [3–5]. However, *A. schindleri,* which was first described in isolates recovered mostly from nonsterile body sites of outpatients, has not been reported in clinically significant isolates from seriously ill hospitalized patients. *A. schindleri* harbouring bla_{NDM-1} recovered during routine groin surveillance from a patient with a blast injury during combat in Afghanistan was previously described [6]; however, the clinical significance of the present isolate was not mentioned.

Here we report the presence of a clinically significant *A. schindleri* (As190) bla_{NDM-1}-positive strain isolated in a 52-year-old woman with a history of positive serology for HIV and hepatitis C virus. The patient also had a history of cocaine addiction, and she had been diagnosed with breast cancer and treated with chemotherapy and radiotherapy. Fifteen days before admission, she sought care for headache, disorientation, vomiting and weakness in the lower limbs. Computed tomographic scan of her brain revealed cystic lesion in the fronto-temporal area, with a midline deviation and perilesional edema. She experienced marked deterioration of consciousness, requiring decompressive craniectomy. During surgery a sample of the cyst was taken for culture; the cultured sample grew *Pseudomonas stutzeri.* The microorganism was sensitive to cefepime, ceftazidime and carbapenems. After being treated with ceftazidime (2 g every 8 hours) for 4 weeks and then discharged with good clinical evolution, she was readmitted to hospital with sensorimotor deterioration, aspiration pneumonia and febrile syndrome. A new computed tomographic scan

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revealed an increased cystic lesion, with increased oedema and midline deviation.

The patient was empirically treated with vancomycin and meropenem, and two blood cultures were performed. At 24 hours' incubation, a Gram-negative bacillus (one of two samples) was isolated which was identified as *Pseudomonas fluorescens* by VITEK 2 (bioMérieux, Marcy l'Étoile, France) and then as *A. schindleri* by matrix-assisted desorption ionization–time of flight mass spectrometry (Bruker Daltonics, Bremen, Germany). This identification was confirmed by *rpoB* gene sequencing (100% homology). Antimicrobial susceptibility testing was performed using VITEK 2, and the results were interpreted according to the 2017 guidelines of the Clinical and Laboratory Standards Institute (Table 1). In order to study the presence of metallo-β-lactamases (MBL), we used a double-disk assay using ethylenediaminetetraacetic acid (EDTA)-sodium mercaptoacetic acid disks (1900/750 g per disk, respectively; Britania Laboratories, Buenos Aires, Argentina) placed 15 mm (centre to centre) from an carbapenem disk (imipenem and meropenem) [7]. An increase ('egg effect') in the inhibition zone of the carbapenem-containing disk near the disk containing the Zn chelating agent (EDTA) was considered to indicate the possible presence of MBLs.

Taking in account these results, we decided to search for the most widespread MBL as well as extended-spectrum β-lactamase genes by PCR amplification. Total DNA extraction was performed according to the manufacturer's instructions (Promega, Madison, WI, USA). We carried out various PCR reactions using previously described primers to determine the presence of bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{SPM}, bla_{PER-2}, bla_{TEM-1}, bla_{GES} and $bla_{CTX-M-2}$ genes [8]. The reactions were performed using the GoTaq enzyme according to the manufacturer's instructions (Promega). We obtained positive results only for the amplification of bla_{NDM-1} in the As190 strain. Nucleotide sequencing and sequence analysis of the positive amplification product showed 99% identity with bla_{NDM-1}. Considering the genes linked to bla_{NDM-1} [1,9], PCR reaction for IS*Aba125* and *aphA6* were performed, giving positive results. Moreover, we performed PCR for other aminoglycoside genes, such as *aac6′-Ib, aacC2* and *aadB1*, and obtained negative results for all of them. To confirm the association among IS*Aba125, aphA6* and bla_{NDM-1}, PCR reactions were performed. Positive PCR reactions were obtained for the following primer combinations: bla_{NDM-1} F-ISAba125F, bla_{NDM-1}R-ISAba125F, bla_{NDM-1}R-aphA6F, aphA6F-ISAba125F and aphA6F-ISAba125R. Sequence analysis confirmed the presence of *aphA6*-IS*Aba125*-bla_{NDM-1} association.

In addition, conjugation assays were performed to see if bla_{NDM-1} could be transferred. Briefly, As190 and *Escherichia coli* J53-2 cells grown with agitation in Luria-Bertani broth were mixed (1:10 and 5:10 donor:recipient) and incubated for 18 hours at 30°C. Cells that may acquire NDM-1 were selected on Luria-Bertani agar with sodium azide (100 μg/mL) and ampicillin (100 μg/mL), and were incubated overnight at 37°C. Negative results were obtained, suggesting that bla_{NDM-1} is present in a nonconjugative element. To find out the exact location of the NDM-1, further studies need to be performed.

Carbapenemases are directly implicated in the increased rates of carbapenem resistance that have been observed lately. Carbapenem-resistant Gram-negative bacilli are of great concern because in some cases no treatment option is available. Continuous surveillance of carbapenem resistance, as well as correct identification at the species level, can contribute in the health system to combat bacterial infections. This report highlights the importance of non-*baumannii Acinetobacter* species harbouring bla_{NDM-1} and the broad dispersion of this carbapenemase among this genus.

TABLE 1. MICs of antimicrobial agents in *Acinetobacter schindleri* isolate

Agent	MIC (μg/mL)	Susceptibility
Ampicillin	≥32	R
Ampicillin/sulbactam	8	S
Cephalothin	≥64	R
Cefotaxime	≥64	R
Ceftazidime	≥64	R
Cefepime	≥64	R
Piperacillin/tazobactam	64	I
Imipenem	≥16	R
Meropenem	≥16	R
Amikacin	≤2	S
Gentamicin	≤1	S
Ciprofloxacin	≤0.25	S
Colistin	≤ 0.5	S
Trimethoprim/sulfamethoxazole	≤2	S

I, intermediate; MIC, minimum inhibitory concentration; R, resistant; S, susceptible.

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Conflict of Interest

None declared.

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