Presence of New Delhi metallo-β-lactamase gene (NDM-1) in a clinical isolate of *Acinetobacter junii* in Argentina

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Keywords: Horizontal gene transfer, nosocomial pathogen, resistance to carbapenem
Original Submission: 8 January 2016; **Revised Submission:** 12 February 2016; **Accepted:** 16 February 2016
Article published online: 23 February 2016

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In the last few years, *Acinetobacter* infections caused by members of this genus other than *baumannii* have been recognized as a result of the implementation of new technologies in diagnostic laboratories. *Acinetobacter junii* is an atypical human pathogen that has been mainly associated with bacteraemia in neonates and paediatric oncology patients. Some cases of meningitis, peritonitis, ocular infection and septicaemia caused by *A. junii* have been reported [1]. Moreover, many recently published reports of *Acinetobacter* spp. harbouring bla_{NDM} suggested *Acinetobacter* as the source and cause of spread for this threatening carbapenemase [2–6]. The identification of bla_{NDM-1} was recently described in *A. junii* clinical isolates from China [6,7].

Here we report the presence of a clinically significant *A. junii* bla_{NDM-1} positive in a 38-year-old woman who was admitted to the emergency department with a fever and leg ulcers with signs of infection. She presented a history of bipolar disorder, hypothyroidism, obesity and chronic necrotizing vasculitis. She had received treatment with corticosteroids and rituximab several months before. Fine-needle puncture aspiration of the ulcers was performed, and empiric treatment with piperacillin/tazobactam at 4.5 g/6 hours was administered intravenously plus vancomycin at 1 g/12 hours administered intravenously.

Aspiration samples from the infected ulcers were cultured and grew *Enterobacter cloacae* after 24 hours of incubation. The isolate was carbapenem susceptible, and the presence of extended β-lactamase activity was detected by Clinical and Laboratory Standards Institute (CLSI) guidelines. Considering the antibiotic susceptibility report, the antimicrobial therapy was changed to ertapenem at 1 g/24 hours, which resulted in the patient recovering well. After 8 days, she became febrile; therefore, the central venous catheter was removed, one blood culture was obtained and vancomycin was added to the antimicrobial therapy.

After 18 hours of incubation, the growth of a Gram-negative, nonfermenting, rod-shaped bacterium, originally identified as *Acinetobacter* spp., was identified from both catheter tip and blood culture samples via conventional biochemical tests. The *Acinetobacter* spp. isolate 23910 was further identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF) MS (Bruker Daltonics), *rpoB* amplification and sequencing to arrive to the species level. MALDI-TOF identified the strain as *A. junii,* with a score of 2.34. This result was confirmed with *rpoB* sequence analysis, which showed 99% identity with *A. junii* strain CIP 107470 (accession no. DQ207483, previously named as *A. grimontii*). The antibiotic susceptibility test was performed using the Phoenix Automated Microbiology System (Becton Dickinson, Franklin Lakes, NJ, USA) using panel NMIC/ID 92 (Gram-negative susceptibility card). The minimum inhibitory concentration (MIC) results were interpreted using the CLSI categories. The MIC results for the tested antibiotics were as followed (μg/mL): ampicillin >16; ampicillin/sulbactam 8/4; piperacillin/tazobactam 16/4; cefazolin >8, cephalotin >16; cefoxitin >16; ceftriaxone >4; ceftazidime >16; cefepime >16; ertapenem >1; imipenem >8; meropenem >8; amikacin $\leq$8; gentamicin $\leq$2; colistin $\leq$1; trimethoprim/sulfamethoxazole $\leq$0.5/9.5; ciprofloxacin 1; levofloxacin $\leq$1; fosfomycin >64. These results revealed that Aj23910 was susceptible to the following: ampicillin/sulbactam, piperacillin/tazobactam, amikacin, gentamicin, colistin, trimethoprim/sulfamethoxazole, ciprofloxacin and levofloxacin. It was resistant to ampicillin, cefazolin, ceftriaxone, cefoxitin, ceftazidime, cefepime, ertapenem, meropenem, imipenem and fosfomycin-G6P.

After the antimicrobial susceptibility report for this strain, the antimicrobial therapy was changed again to ampicillin/sulbactam at 1.5 g/6 hours administered intravenously. The clinical finding—the same as *A. junii* isolate recovered from the blood

http://dx.doi.org/10.1016/j.nmni.2016.02.008

culture and from the catheter tip culture—was interpreted as a bacteraemia associated with venous central catheter.

In order to test for the presence of metallo-β-lactamase (MBL), we performed disk diffusion assays and a double-disk assay using an EDTA/SMA disk (1900/750 μg per disk, respectively) (Laboratorios Britania, Buenos Aires, Argentina) and an imipenem disk (placed 15 mm from each other). This assay showed synergism between carbapenem and EDTA/SMA disks, which suggests the presence of a putative MBL present in Aj23910. Considering the results, we decided to search for the most widespread MBL genes by PCR amplification. Total DNA extraction was performed according to the manufacturer's instructions (Promega, Madison, WI, USA). We carried out PCR reactions using previously described primers to determine the presence of bla_{VIM}, bla_{IMP}, bla_{NDM} and bla_{SPM} genes [4]. The reactions were performed using GoTaq enzyme according to the manufacturer's instructions (Promega). We obtained positive results for the amplification of bla_{NDM-1} in the Aj23910 strain. Nucleotide sequencing and sequence analysis of the positive amplification showed 100% identity with bla_{NDM1}. We also obtained positive results for IS*Aba125* and *aphA6* genes, which were previously reported in the same genetic context as NDM [3,4,8]. PCR reactions revealed the link and proximity of these genes to bla_{NDM-1}. Positive PCR products (bla_{NDM-1} F-ISAba125F, bla_{NDM-1} R-ISAba125F, bla_{NDM-1} R-aphA6F, aphA6F- ISAba125F and aphA6F-ISAba125R) were sequenced. The sequence analyses confirmed the presence of aphA6-ISAba125–bla_{NDM-1} association.

In addition, conjugation assays were performed to see if bla_{NDM-1} was plasmid located. Briefly, Aj23910 and *Escherichia coli* J53-2 cells grown with agitation in Luria Bertani (LB) broth were mixed (1:10 and 5:10 donor:recipient) and incubated for 18 hours at 30°C. Transconjugant cells were selected on LB agar supplemented with sodium azide (150 μg/mL) and ampicillin (100 μg/mL) and were incubated overnight at 37°C. The negative results from the conjugation assay suggests that bla_{NDM-1} is codified in a nonconjugative element. Further studies are required to determine whether the gene possessed a chromosome location or a nonconjugative element location.

The NDM-1 carbapenemase has been dramatically spread among Gram-negative bacilli, thus imposing a new challenge on the health system to fight bacterial infections.

These data expand the number of *Acinetobacter* species harbouring bla_{NDM-1}. The wide existence of *Acinetobacter* harbouring and dispersing this carbapenemase emphasizes the importance of non–previously recognized pathogens as reservoirs of dangerous resistance determinants. These resistance determinants can be later easily transferred to other menacing pathogens.

Acknowledgements

Supported by grants from the Secretaría de Ciencia y Técnica de la Universidad de Buenos Aires (UBACyT, ANPyCT PICT 0212) to C. Vay and PICT 0120 to MSR, Buenos Aires, Argentina. MSR is a member of the Consejo Nacional de Investigaciones Científicas y Técnicas (CONICET) research career, and SM has a doctoral fellowship from CONICET.

Conflict of Interest

None declared.

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