Pathology (■ xxxx) **xxx(xxx)**, xxx

CORRESPONDENCE

Isolation of *Acinetobacter bereziniae* harbouring plasmid *bla*$_{NDM-1}$ in central Sydney, Australia

To the Editor,

We report the isolation of a multidrug resistant *Acinetobacter bereziniae* isolate harbouring a plasmid *bla*$_{NDM-1}$ in central Sydney, Australia from a patient who had travelled to the Philippines. While carbapenem-resistant *Acinetobacter baumannii* complex (e.g., *A. baumannii, A. calcoaceticus, A. baumannii, A. dijkshoorniae, A. nosocomialis, A. pittii and A. seifertii)* (CRAB) group have been previously described, carrying the *bla*$_{NDM-1}$,[1–3] only a few cases of *A. bereziniae* clinical isolation have been described worldwide carrying such resistance.[4,5] *Acinetobacter berezinae* is an environmental *Acinetobacter* species of the non-*baumanii* complex, with some routine medical biochemical databases such as the Vitek 2 XL unable to identify it correctly. Conversely, matrix-assisted laser desorption ionisation time of flight mass spectrometry (MALDI-TOF MS) offers a rapid and convenient identification method for routine laboratories,[6] and has been shown here to accurately identify an *A. bereziniae* isolate as confirmed by molecular methods. Furthermore, *A. bereziniae* harbouring a plasmid *bla*$_{NDM-1}$ described here highlights the potential role of non-*baumannii Acinetobacter* disseminating antibiotic resistance genes through hospital environments.[7]

The *Acinetobacter bereziniae* isolate carrying the metallo-β-lactamase resistance gene was isolated from the rectal swabs collected during infection control screening after the patient's recent hospitalisation in the Philippines. The organism did not grow on ESBL Brilliance agar (ThermoFisher, Australia) but grew on CRE Brilliance agar (ThermoFisher) as clear, slightly pigmented yellow colonies incubated 24−48 h at room temperature as instructed by the manufacturer (Fig. 1). The organism growing on the selective CRE Brilliance agar media was a Gram-negative coccobacillus by Gram stain and sub-cultured on horse blood agar (ThermoFisher) for further identification and susceptibility testing. The organism was first identified as *Acinetobacter bereziniae* by MALDI-TOF MS (Bruker, Australia) with a score of 2.53. This was a good reliable result using this identification system since a score value $\geq$2 indicated species identification; a score value between 1.7 and 1.9 indicated genus identification, and a score value $<$1.7 indicated no identification. Identification using the Vitek 2 XL (bioMerieux, Australia) system was also performed by using the GNI ID card with reference code 21341 (bioMerieux). After 9.92 hours, the automated identification system gave an excellent identification as *Acinetobacter lwoffii* with a probability of 99% with a Bionumber of 0001011101500102. The identification as *Acinetobacter bereziniae* was later confirmed using 16S rRNA sequencing. Results showed a 100% match using sequence data assembled and compared with previously reported sequence by using the basic local alignment search tool (BLAST) of the National Centre for Biotechnology Information (NCBI) database.

On further testing the RAPIDEC CARBA NP (bioMerieux) was positive for the isolate which was indicative of the presence of a carbapenemase gene. Molecular testing using the gene Xpert Carba-R (Cepheid, Australia), a real-time polymerase chain reaction assay for rapid detection and differentiation of five genes (*bla*$_{KPC}$, *bla*$_{VIM}$, *bla*$_{OXA-48}$, *bla*$_{IMP-1}$, *bla*$_{NDM}$) revealed the presence of a *bla*$_{NDM}$ carbapenemase gene. The isolate was later referred to a reference laboratory, the Institute of Clinical Pathology and Medical Research (ICPMR) in Westmead Hospital, Sydney, for whole genome sequencing which confirmed the presence of *bla*$_{NDM-1}$ and further classified it as ST1318. Carbapenem resistance and further susceptibility of the organism to other antibiotics were confirmed using Vitek 2 XL, Etest strips (bioMerieux) and disc-diffusion (Thermofisher) susceptibility testing using both European Committee on Antimicrobial Susceptibility Testing (EUCAST) and Clinical and Laboratory Standards Institute (CLSI) guidelines.[8,9] Colistin susceptibility was confirmed by broth microdilution (BMD) using the MICRONAUT MIC-Strip (MERLIN Diagnostika, Dutec, Australia). BMD quality control was performed with both *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (*mcr-1* positive). *Acinetobacter bereziniae* antibiotic susceptibility results are shown in Table 1.

Acinetobacter species are ubiquitous in nature, and their ability to survive in many ecological niches is worrisome, especially when carrying multidrug-resistant genes in a hospital environment. *Acinetobacter bereziniae*, also referred to as *Acinetobacter genospecies* 10, has been isolated in sewage in Denmark, environmental surfaces in Korea, vegetables in Hong Kong and UK, meat and animals in Lebanon, as well as human skin in Germany and Hong Kong.[7] The first *A. bereziniae* carrying the *bla*$_{NDM-1}$ gene was reported in Brazil in 2014 from a 69-year-old HIV-positive man admitted to ICU due to respiratory illness.[4] Other cases include a bacteraemia in Argentina from a 53-year-old patient who had undergone chemotherapy due to leukaemia,[5] and cases of this organism carrying other metallo-β-lactamase resistances such as *bla*$_{NDM}$, *bla*$_{IMP}$ and *bla*$_{VIM}$ genes have also been reported in Japan.[10,11] To our knowledge no one has previously reported the isolation of *A. bereziniae* carrying metallo-β-lactamase-resistant *bla*$_{NDM-1}$ within the central Sydney region.

Phenotypic identification of non-*Acinetobacter* species is problematic and challenging, as shown here with *A. bereziniae* misidentified as *A. lwoffi* from our Vitek 2 identification results, and also described by other studies with the Vitek MS and MicroScan.[6] MALDI-TOF MS offers a rapid, convenient identification method for diagnostic laboratories, and can be coupled with other molecular techniques such as 16S rRNA sequencing, *rpoB*, ARDRA, SDS-PAGE, ribotyping, DNA-DNA hybridisation, RAPD or whole genome sequencing[6,7] provided by reference laboratories.

It should be noted that the current National Alert System for Critical Antimicrobial Resistances (CARAlert) handbook[2] in Australia lists *Acinetobacter baumannii* complex species such as *A. calcoaceticus, A. baumannii, A. dijkshoorniae, A. nosocomialis, A. pittii* and *A. seifertii*, as potential organisms carrying carbapenem resistance; however,

Print ISSN 0031-3025/Online ISSN 1465-3931

Please cite this article as: Merlino J et al., Isolation of *Acinetobacter bereziniae* harbouring plasmid *bla*$_{NDM-1}$ in central Sydney, Australia, Pathology, https://doi.org/10.1016/j.pathol.2023.02.008

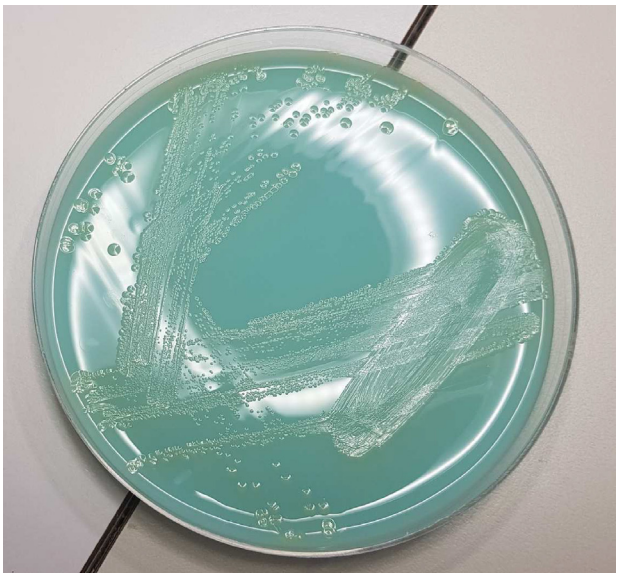

Fig. 1 *Acinetobacter bereziniae* carrying the bla_{NDM-1} plasmid gene growing on CRE Brilliance agar (Thermofisher) as clear, slightly pigmented yellow colonies incubated 24−48 h at room temperature.

Table 1 Antibiotic resistant profile of the *Acinetobacter bereziniae* carrying the bla_{NDM-1} gene

Antibiotic	MIC	EUCAST	CLSI
Meropenem[a/b]	8	I	R
Imipenem[a]	6	R	R
Ciprofloxacin[a]	>34	R	R
Piperacillin/tazobactam[a]	12	−	I
Cefepime[b]	>64	−	R
Gentamicin[b]	4	S	S
Amikacin[a]	32	R	R
Tetracyline[a]	24	−	R
Tigecycline[a]	1	−	−
Aztreonam[a]	>64	−	−
Colistin[c]	2	S	S

CLSI, Clinical and Laboratory Standards Institute; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration.
Interpretations: I, intermediate; R, resistant; S, susceptible; −, no breakpoints.
[a] MIC determined by Etest strips.
[b] MIC determined by Vitek 2XL ASTN246 card.
[c] MIC confirmed by BMD quality control performed with both *E. coli* ATCC 25922 and *E. coli* NCTC 13846 (*mcr-1* positive).

there is also the worrisome potential role of other non-*baumannii Acinetobacter* species such as *A. bereziniae* to carry carbapenem-resistant genes which have implications in infection control in hospital environments. *Acinetobacter bereziniae* carrying the bla_{NDM-1} have been shown to carry IS*Aba* elements very similar to multidrug-resistant *A. baumannii* isolates, with the potential ability to mobilise as a whole and act as reservoirs of bla_{NDM} genes which may contribute to their spreading among clinically relevant Enterobacterales.[12]

Acknowledgement: We thank Dr Andrew Ginn, the Institute of Clinical Pathology and Medical Research (ICPMR), Westmead Hospital, Sydney for whole genome sequencing of the isolate and confirming the presence of bla_{NDM-1}.

Conflicts of interest and source of funding: The authors state there are no conflicts of interest to disclose.

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DOI: https://doi.org/10.1016/j.pathol.2023.02.008

Please cite this article as: Merlino J et al., Isolation of *Acinetobacter bereziniae* harbouring plasmid bla_{NDM-1} in central Sydney, Australia, Pathology, https://doi.org/10.1016/j.pathol.2023.02.008