

# First Report of *bla*<sub>NDM</sub> and *bla*<sub>OXA-58</sub> Coexistence in *Acinetobacter junii*

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*Acinetobacter* species are a major cause of hospital-acquired infections, especially in intensive care units (ICUs) (1). This, in part, is due to the bacteria's ability to survive long-term on hospital environment surfaces and their ability to acquire antibiotic resistance genes (2). One of the *Acinetobacter* species most often associated with high morbidity and mortality is *A. baumannii* (1). However, other *Acinetobacter* species have also been associated with acquiring broad-spectrum antibiotic resistance genes and causing severe disease (3–6). Here we report the isolation of an *A. junii* strain that was resistant to all β-lactam antibiotics, including the carbapenems, and the aminoglycosides. This isolate's carbapenem resistance mechanism was a result of acquiring two carbapenem resistance genes, *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-58</sub>. To our knowledge, this is the first report of the coexistence of *bla*<sub>NDM-1</sub> and *bla*<sub>OXA-58</sub> in *A. junii*.

The *A. junii* strain was isolated from surveillance swabs collected from a 5-day-old baby girl who was referred to Caritas Baby Hospital (CBH) on 9 October 2012 from another hospital located in the northern part of Palestine. Upon admission to CBH's pediatric ward, surveillance (nose, rectal, and umbilical) swabs were collected from the patient on Copan Transystem swabs (Copan Diagnostics, Corona, CA) and transferred to the clinical laboratory within 30 min. This is part of the infection control policies that were followed at CBH to evaluate the presence of bacteria resistant to broad-spectrum antibiotics in high-risk patients referred from other medical institutions. The Caritas Baby Hospital Medical Research Committee approved the study (MRC-14).

The specimens were inoculated on a selective agar medium (MacConkey-cefotaxime [10 μg/ml] and MacConkey-meropenem [0.5 μg/ml]) and incubated for 24 h at 37°C as previously described (7, 8). A weakly lactose-fermenting, oxidase-negative, Gram-negative coccobacillus was isolated on both selective media and was presumptively identified as *Acinetobacter* species. The identification of the isolate as *A. junii* was obtained after sequencing the first 500 bp of the amplified 16S rRNA gene as previously described (9). Antimicrobial susceptibility testing was performed by disk diffusion (Oxoid, United Kingdom) according to the standards of the Clinical and Laboratory Standards Institute (10). For the antimicrobial agent colistin, we used the Etest (AB Biodisk; bioMérieux, France) to determine its MIC. The antibiogram showed a total resistance to all β-lactam antibiotics and the aminoglycosides (Table 1). On the other hand, the isolate was susceptible to the fluoroquinolones, tetracyclines, trimethoprim-sulfamethoxazole, and colistin (Table 1).

In order to identify the carbapenem resistance mechanism that mediated *A. junii*'s resistance, PCR using primers for the class A carbapenemases (*bla*<sub>NDM</sub>, *bla*<sub>SME</sub>, *bla*<sub>IMP</sub>, *bla*<sub>GES</sub>, and *bla*<sub>KPC</sub>), the class D oxacillinases (*bla*<sub>OXA-58-like</sub>, *bla*<sub>OXA-23-like</sub>, *bla*<sub>OXA-24-like</sub>, *bla*<sub>OXA-51-like</sub>, *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-24</sub>, *bla*<sub>OXA-69</sub>, *bla*<sub>OXA-48</sub>, *bla*<sub>OXA-50</sub>, and *bla*<sub>OXA-60</sub>), and the class B metalloenzymes (*bla*<sub>IMP-1</sub>, *bla*<sub>IMP-2</sub>

TABLE 1 Susceptibility patterns of the *bla*<sub>NDM</sub>- and *bla*<sub>OXA-58</sub>-positive *A. junii* isolate<sup>a</sup>

Antibiotic(s) (concn [μg])	Susceptibility
Mezlocillin (75)	R
Piperacillin-tazobactam (100, 10)	R
Ceftazidime (30)	R
Cefepime (30)	R
Cefotaxime (30)	R
Ceftriaxone (30)	R
Imipenem (10)	R
Meropenem (10)	R
Amikacin (30)	R
Gentamicin (10)	R
Ciprofloxacin (5)	S
Levofloxacin (5)	S
Trimethoprim-sulfamethoxazole (1.25, 23.75)	S
Tetracycline (30)	S

<sup>a</sup> The isolate identification number is 132390. The colistin MIC was 0.125 μg/ml. R, resistant; S, susceptible.

*bla*<sub>VIM-1</sub>, *bla*<sub>VIM-2</sub>, *bla*<sub>SPM-1</sub>, *bla*<sub>GIM-1</sub>, *bla*<sub>NDM</sub>, and *bla*<sub>SIM-1</sub>) was performed on the isolate's nucleic acid as previously reported (8, 11, 12). PCR amplification from the *A. junii* isolate nucleic acid gave the appropriate PCR product for *bla*<sub>NDM</sub> and *bla*<sub>OXA-58</sub>. The *bla*<sub>NDM</sub> and *bla*<sub>OXA-58</sub> PCR products were sequenced using the BigDye Terminator v3.1 cycle sequencing kit (Life Technology, USA) to confirm the identities of both genes. This represents the first report of *A. junii* positive for both the *bla*<sub>OXA-58</sub> and the *bla*<sub>NDM</sub> carbapenem resistance genes.

Carbapenem resistance mediated by either the *bla*<sub>NDM</sub> or the *bla*<sub>OXA-58</sub> gene has been reported before in *A. junii* (6, 13). The *bla*<sub>NDM</sub> gene was detected in an *A. junii* isolate from China. This isolate was resistant to the β-lactam antibiotics but sensitive to the aminoglycosides and the fluoroquinolones (13). On the other hand, *bla*<sub>OXA-58</sub>-positive *A. junii* isolates were detected in Romania and Australia (6, 14). The characterized isolate from Australia has an antibiotic resistance profile similar to the one detected in Palestine. It was resistant to all β-lactam antibiotics and gentamicin but sensitive to the fluoroquinolones, amikacin, tetracycline, and polymyxin B (6).

The isolation of carbapenem-resistant *A. junii* with combined antibiotic resistance mechanisms suggests that surveillance swabs

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collected from high-risk patients play an important role in controlling the spread of drug-resistant bacteria.

**Nucleotide sequence accession numbers.** The first 500 bp of the amplified 16S rRNA gene of our isolate was deposited in GenBank under accession number [KJ024808](https://doi.org/10.1016/j.ccc.2007.12.005). Its *bla*<sub>OXA-58</sub> and the *bla*<sub>NDM</sub> sequences were deposited under accession numbers [KJ024809](https://doi.org/10.1016/j.ccc.2007.12.005) and [KJ024810](https://doi.org/10.1016/j.ccc.2007.12.005).

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#### REFERENCES

- Murray CK, Hospenthal DR. 2008. Acinetobacter infection in the ICU. *Crit. Care Clin.* 24:vii, 237–248. <http://dx.doi.org/10.1016/j.ccc.2007.12.005>.
- Bergogne-Berezin E, Towner KJ. 1996. Acinetobacter spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. *Clin. Microbiol. Rev.* 9:148–165.
- Bonnin RA, Cuzon G, Poirel L, Nordmann P. 2013. Multidrug-resistant Acinetobacter baumannii clone, France. *Emerg. Infect. Dis.* 19:822–823. <http://dx.doi.org/10.3201/eid1905.121618>.
- Giannouli M, Cuccurullo S, Crivaro V, Di Popolo A, Bernardo M, Tomasone F, Amato G, Brisse S, Triassi M, Utili R, Zarrilli R. 2010. Molecular epidemiology of multidrug-resistant Acinetobacter baumannii in a tertiary care hospital in Naples, Italy, shows the emergence of a novel epidemic clone. *J. Clin. Microbiol.* 48:1223–1230. <http://dx.doi.org/10.1128/JCM.02263-09>.
- Linde HJ, Hahn J, Holler E, Reischl U, Lehn N. 2002. Septicemia due to Acinetobacter junii. *J. Clin. Microbiol.* 40:2696–2697. <http://dx.doi.org/10.1128/JCM.40.7.2696-2697.2002>.
- Peleg AY, Franklin C, Walters LJ, Bell JM, Spelman DW. 2006. OXA-58 and IMP-4 carbapenem-hydrolyzing beta-lactamases in an Acinetobacter junii blood culture isolate from Australia. *Antimicrob. Agents Chemother.* 50:399–400. <http://dx.doi.org/10.1128/AAC.50.1.399-400.2006>.
- Al-Dawodi RL, Ghneim R, Kattan R, Siryani R, Abu-Diab I, Ghneim A, Zoughbi R, Issa M, Al Qass A, Turkuman R, Marzouqa SH, Hindiyyeh M. 2013. Evaluation of meropenem, imipenem and eropenem impregnated MacConkey agar plates for the detection of carbapenem resistant Enterobacteriaceae. *Int. Arab. J. Antimicrob. Agents* 3:5.
- Kattan RL, Ghneim R, Siryani R, Al-Dawodi I, Abu-Diab R, Ghneim A, Zoughbi R, Issa M, Al Qass A, Turkuman R, Corradin S, Marzouqa LH, Hindiyyeh M. 2012. Emergence of Klebsiella pneumoniae carbapenemase (*bla*<sub>KPC-2</sub>) in members of the Enterobacteriaceae family in Palestine. *Int. Arab. J. Antimicrob. Agents* 2:4.
- Janda JM, Abbott SL. 2007. 16S rRNA gene sequencing for bacterial identification in the diagnostic laboratory: pluses, perils, and pitfalls. *J. Clin. Microbiol.* 45:2761–2764. <http://dx.doi.org/10.1128/JCM.01228-07>.
- CLSI. 2013. Performance standards for antimicrobial susceptibility testing. Sixteenth informational supplement. CLSI, Wayne, PA.
- Huang J, Wang M, Ding H, Ye M, Hu F, Guo Q, Xu X, Wang M. 2013. New Delhi metallo-beta-lactamase-1 in carbapenem-resistant Salmonella strain, China. *Emerg. Infect. Dis.* 19:2049–2051. <http://dx.doi.org/10.3201/eid1912.130051>.
- Queenan AM, Bush K. 2007. Carbapenemases: the versatile beta-lactamases. *Clin. Microbiol. Rev.* 20:440–458. <http://dx.doi.org/10.1128/CMR.00001-07>.
- Zhou WQ, Zhang ZF, Shen H, Ning MZ, Xu XJ, Cao XL, Zhang K. 2013. First report of the emergence of New Delhi metallo-beta-lactamase-1 producing Acinetobacter junii in Nanjing, China. *Indian J. Med. Microbiol.* 31:206–207.
- Marque S, Poirel L, Heritier C, Brisse S, Blasco MD, Filip R, Coman G, Naas T, Nordmann P. 2005. Regional occurrence of plasmid-mediated carbapenem-hydrolyzing oxacillinase OXA-58 in Acinetobacter spp. in Europe. *J. Clin. Microbiol.* 43:4885–4888. <http://dx.doi.org/10.1128/JCM.43.9.4885-4888.2005>.