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Molecular characterization of NDM-1-producing *Acinetobacter pittii* isolated from Turkey in 2006

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Sir,

In Europe, bla_{NDM-1} has been found in the chromosome of a few *Acinetobacter baumannii* isolates typically linked with patient travel to North Africa or the Balkans, while in south Asia and China in particular, NDM is frequently found in different *Acinetobacter* spp. and it is usually plasmid encoded.[1]

In this study, we report the earliest occurrence of NDM-1 in *Acinetobacter pittii*. The isolate was recovered from a female patient in her late teens from a small city in eastern Turkey, who was transferred to Hacettepe University with a presumptive diagnosis of peritoneal and meningeal carcinomatosis. The patient underwent surgery for metastatic carcinoma (Krukenberg tumour) in early 2006 and had no history of travel outside of her home town except for travel to Ankara for her current medical treatment during the preceding month. During the subsequent 4 months of hospitalization, the patient experienced several febrile neutropenic episodes due to chemo- and radiotherapy, for which she was treated with piperacillin/tazobactam, imipenem, meropenem, metronidazole, teicoplanin, vancomycin and amphotericin B lipid complex at different times.

By the end of the fourth month, a blood culture was positive for *Acinetobacter*, initially reported as *A. baumannii*, but later identified as *A. pittii* by MALDI-TOF MS and amplified ribosomal DNA restriction analysis.[2,3] Antibiotic susceptibility testing performed by disc diffusion or Etest and interpreted according to EUCAST guidelines (version 4.0, 2014; http://www.eucast.org) showed resistance to carbapenems and amikacin and susceptibility to gentamicin, tobramycin, netilmicin, ciprofloxacin and colistin (Table 1). She was given cefoperazone/sulbactam (1/1 g twice daily) and netilmicin (130 mg once daily) before the susceptibility results were available. The patient rapidly defervesced and this treatment was continued for 14 days. A follow-up culture on the third day of therapy did not yield any microbial growth. Upon completion of therapy, the patient was discharged.

The *A. pittii* isolate (designated as JVAP02) yielded positive results when using either the cloverleaf test (modified Hodge test) or imipenem-EDTA Etest strips to screen for carbapenemase/metallo-β-lactamase production. PCR screening for β-lactamase genes followed by DNA sequencing identified the presence of bla_{NDM-1}, but was negative for bla_{OXA-51}, bla_{OXA-23}, bla_{OXA-24}, bla_{OXA-58}, bla_{VIM} or bla_{IMP}.

MLST performed according to the MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst/) identified four new alleles (*cpn60*:66, *pyrG*:35, *recA*:72 and *rpoB*:62) and strain JVAP02 was assigned a novel sequence type, ST457, by the Pasteur curators.

S1-PFGE and Southern hybridization with a digoxigenin-labelled probe against bla_{NDM-1} demonstrated the genetic location of bla_{NDM-1} within a plasmid of ~45 kb, which could not be assigned to any plasmid replicon group using the PCR-based replicon typing scheme devised for *A. baumannii*.[4]

Characterization of the genetic structures surrounding the bla_{NDM-1} gene was performed by inverse PCR and PCR mapping revealing that bla_{NDM-1} was flanked by two IS*Aba125* forming a canonical Tn*125* composite transposon identical to that of the pNDM-BJ01 plasmid from *Acinetobacter lwoffii*.[5] Of note, the downstream IS*Aba125* was inserted within the *delta-pac* gene at the same location where alternative ISs have been described in other NDM-producing non-*baumannii Acinetobacter* isolates,[6–8] suggesting this might be a hotspot for other ISs. Upstream from bla_{NDM-1}, we also found the *aphA6* gene encoding amikacin resistance as well as the two additional ISs reported in pNDM-BJ01.[5] PCR assays for methylase-encoding genes *rmtA*, *rmtB*, *rmtC*, *rmtD*, *armA* and *npmA* as well as genes encoding additional aminoglycoside-modifying enzymes were negative.

Transferability studies were performed by biparental mating assays and DNA electroporation using *Escherichia coli* MC1061Rif^r and *A. baumannii* ATCC 17978Rif^r strains as recipients. *A. baumannii* ATCC 17978Rif^r transconjugants and transformants harbouring bla_{NDM-1} and *aphA6* were successfully selected on LB agar plates containing imipenem (16 mg/L) and rifampicin (75 mg/L), but bla_{NDM-1} could not be transferred into *E. coli* MC1061Rif^r even when using imipenem concentrations of 1 mg/L. Plasmid acquisition yielded *A. baumannii* ATCC 17978Rif^r resistant to carbapenems, but was only able to produce reduced susceptibility to kanamycin and amikacin (Table 1), suggesting reduced expression of the *aphA6* gene as previously noted.[5]

To our knowledge, this is the first NDM-producing *Acinetobacter* isolate described in Turkey and also one of the earliest NDM producers overall (the earliest report of NDM in *E. coli* dates from 2006 as well).[9] The origin of NDM-1-positive *A. pittii* in Turkey remains unclear, since the patient had no history of travel to geographical areas known to be affected by NDM. Interestingly, the genetic context as well as the genetic platform harbouring NDM-1 in the current isolate (plasmid size of ~45 kb, undetermined replicon type and non-functional *aphA6* gene) shares several features

Table 1. Antimicrobial susceptibility of *A. baumannii* ATCC 17978Rif^r, *A. pittii* JVAP02, an *A. baumannii* ATCC 17978Rif^r transformant and an *A. baumannii* ATCC 17978Rif^r transconjugant expressing NDM-1

	MIC (mg/L)			
Antimicrobial agent	*A. baumannii* ATCC 17978Rif^r	*A. pittii* JVAP02	*A. baumannii* ATCC 17978Rif^r-T	*A. baumannii* ATCC 17978Rif^r-C
Imipenem	0.25	>32	>32	>32
Meropenem	0.38	>32	>32	>32
Ertapenem	1.5	>32	>32	>32
Cefotaxime	6.0	>256	>256	>256
Ceftazidime	3.0	>256	>256	>256
Cefepime	2.0	>256	>256	>256
Cefoperazone/ sulbactam	ND	>256	ND	ND
Aztreonam	12	12	12	12
Gentamicin	0.19	3.0	0.5	0.25
Amikacin	0.38	24	2.0	2.0
Kanamycin	0.5	>256	6.0	8.0
Netilmicin	ND	1	ND	ND
Tobramycin	0.125	2.0	0.38	0.125
Colistin	0.125	0.19	0.38	0.38
Ciprofloxacin	0.5	0.5	0.38	0.5
Tetracycline	4.0	8.0	3.0	4.0
Tigecycline	0.5	1.5	0.75	0.75
Rifampicin	>32	4.0	>32	>32

T, *A. baumannii* ATCC-derived transformant; C, *A. baumannii* ATCC-derived transconjugant; ND, not determined.

with those of non-*baumannii Acinetobacter* isolates from China, Afghanistan and Paraguay,[5–8,10] suggesting that NDM enzymes may have been circulating among non-*baumannii Acinetobacter* spp. in other parts of the world prior to their major spread to more clinically relevant bacterial species.

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Transparency declarations

None to declare.

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