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# Isolation of Genetically Unrelated $bla_{NDM-1}$-Positive *Providencia rettgeri* Strains in Israel

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The emergence of the novel carbapenem resistance mechanism New Delhi metallo-β-lactamase ($bla_{NDM-1}$) in *Enterobacteriaceae* has presented a new challenge to health care providers on how to deal with infected or colonized patients. In most cases, the $bla_{NDM-1}$ gene is borne on large plasmids; however, it has also been reported to be chromosomally integrated in some bacterial strains (1). $bla_{NDM-1}$ was first described in 2009 in a *Klebsiella pneumoniae* isolated in a Swedish hospital from a patient previously hospitalized in a medical institution in India (2). Since then, the isolation of bacterial species carrying the $bla_{NDM-1}$ gene has been reported in several countries, usually from patients who traveled to the Indian subcontinent (3–6).

Here, we report the isolation of 5 *Providencia rettgeri* strains positive for the $bla_{NDM-1}$ gene from Israeli patients with no history of travel outside Israel. The *P. rettgeri* strains were isolated from one patient in 2008 and from 4 patients in 2011 (Tables 1 and 2). Identification of all 5 isolates to the genus and species levels was performed using the BD Phoenix test (BD, NJ, USA) and confirmed by 16S rRNA sequencing. All 5 isolates tested positive for metallo-β-lactamase using the Etest MBL (bioMérieux, Solna, Sweden). The presence of metallo-β-lactamase enzymes were further confirmed by PCR amplification and sequencing of the $bla_{NDM}$ gene from all five of the *P. rettgeri* isolates.

Pulse-field gel electrophoresis (PFGE) after SpeI digest of the isolates' DNA was performed and interpreted as previously described by Tenover et al. (7). Three PFGE patterns were detected. Three strains displayed genetic similarities but were not identical (patterns D1 to D3), while two isolates were genetically distinct and were categorized as nonclonal (patterns E and F) (Table 1). This finding was of considerable significance, as it indicated that our institution was not experiencing an outbreak of genetically related *P. rettgeri*. Further analysis of the $bla_{NDM-1}$-producing isolates revealed that the $bla_{NDM-1}$ resistance mechanism is most likely chromosomally integrated, since conjugation studies failed to transfer the $bla_{NDM-1}$ resistance mechanism from the 5 *P. rettgeri* isolates to a susceptible azide-resistant plasmid-less *Escherichia coli* J5-3 strain, as was previously described (8). In addition, transformation of extracted plasmid DNA from the *P. rettgeri* strains into electrocompetent *E. coli* MC1022 failed to transfer the ampicillin resistance phenotype and did not transfer the $bla_{NDM-1}$ resistance mechanism either.

**TABLE 1** Summary of the characteristics of the 5 $bla_{NDM-1}$ positive *P. rettgeri* strains

| *P. rettgeri* isolate | Sample Source | Result by: Modified Hodge test | Etest MBL | Conjugation studies | Transformation studies | PFGE pulsotype |
|---|---|---|---|---|---|---|
| 34445/11 | Blood | Negative | Positive | Negative | Negative | D1 |
| 23748/11 | Blood | Negative | Positive | Negative | Negative | D2 |
| 3433/11 | Rectal | Negative | Positive | Negative | Negative | D3 |
| 5932/08 | Pus | Negative | Positive | Negative | Negative | E |
| 32649/11 | Blood | Negative | Positive | Negative | Negative | F |

**TABLE 2** Summary of the antimicrobial resistance profiles of the 5 $bla_{NDM-1}$ positive *P. rettgeri* strains[a]

| Antimicrobial agent | MIC(s) (mg/liter) for *P. rettgeri* isolate: 34445/11 | 23748/11 | 3433/11 | 5932/08 | 32649/11 |
|---|---|---|---|---|---|
| Ampicillin-sulbactam | >16, 8 | >16, 8 | >16, 8 | >16, 8 | >16, 8 |
| Amoxicillin-clavulanate | >16, 8 | >16, 8 | >16, 8 | >16, 8 | >16, 8 |
| Piperacillin-tazobactam | 64, 4 | >64, 4 | 32, 4 | >64, 4 | 64, 4 |
| Ceftriaxone | 4 | 4 | 32 | >16 | >16 |
| Ceftazidime | >16 | >16 | >16 | >16 | >16 |
| Cefepime | 2 | ≤1 | ≤1 | ≤1 | >16 |
| Aztreonam | <2 | <2 | <2 | >16 | <2 |
| Gentamicin | >8 | >8 | >8 | >8 | 8 |
| Amikacin | >32 | >32 | <8 | <8 | 16 |
| Trimethoprim-sulfamethoxazole | >4, 76 | >4, 76 | >4, 76 | >4, 76 | >4, 76 |
| Ciprofloxacin | >2 | >2 | >2 | >2 | >2 |
| Norfloxacin | >8 | >8 | >8 | >8 | >8 |
| Cefoxitin | 16 | >16 | >16 | >16 | >16 |
| Ertapenem (Etest) | 4 | 0.5 | 4 | >32 | >32 |
| Meropenem (Etest) | >32 | 4 | 16 | >32 | >32 |
| Imipenem (Etest) | >32 | >32 | >32 | >32 | >32 |
| Colistin (Etest) | >256 | >256 | >256 | >256 | >256 |
| Tigecycline (Etest) | 4 | 64 | 4 | 64 | 2 |

[a] MICs were determined by the BD Phoenix UNMIC/ID-83 card or the Etest, as indicated. Where a ">" sign is present in an entry with two values, the sign applies to both values in the entry. The second MIC refers to the second drug in the compound.

Antimicrobial susceptibility testing of the 5 *P. rettgeri* strains, which was performed according to CLSI guidelines (9) with very limited treatment options, showed different and extreme resistance profiles (Table 2). Various MICs were found for the different carbapenems tested by the Etest (bioMérieux, Marcy l'Etoile, France) (Table 2). Interestingly, the MICs of tigecycline for two of the 5 *P. rettgeri* isolates were high (64 mg/liter), which further complicated patient management, while the MIC values for the others were between 2 and 4 mg/liter.

We are the first to report the isolation and detection of 5 non-

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genetically related *P. rettgeri* $bla_{NDM-1}$ isolates in an Israeli medical institution from patients with no previous travel history outside Israel. These isolates did not spread in the hospital setting, which could be in part due to the extensive infection control measures that were implemented at our institution following the $bla_{KPC}$ outbreak in 2006. The emergence of these extremely drug resistant bacteria is a reminder of the importance of mandating prudent usage of antibiotics and implementing infection control measures to control the spread of these resistant strains.

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