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# Letters to the Editor

## RmtC 16S rRNA Methyltransferase in Australia▽

*Proteus mirabilis* isolate JIE273 was recovered in March 2007 from the urine of an inpatient at Blacktown Hospital, Sydney. In vitro susceptibility tests (Phoenix NMIC/ID-101 panel; BD, Franklin Lakes, NJ) indicated that JIE273 was susceptible to imipenem (MIC, ≤2 μg/ml), cefoxitin (MIC, ≤4 μg/ml), amoxicillin-clavulanate (MIC, ≤4/2 μg/ml), and ticarcillin-clavulanate (MIC, ≤2/2 μg/ml). It had "intermediate" susceptibility to ticarcillin (MIC, 32 μg/ml) but was resistant to ampicillin (MIC, >16 μg/ml), cephalothin (MIC, >16 μg/ml), cefuroxime (MIC, >16 μg/ml), cefotaxime (MIC, >32 μg/ml), ceftriaxone (MIC, >32 μg/ml), ceftazidime (MIC, >16 μg/ml), aztreonam (MIC, >16 μg/ml), trimethoprim-sulfamethoxazole (MIC, >2/38 μg/ml), ciprofloxacin (MIC, >2 μg/ml), levofloxacin (MIC, >4 μg/ml), and nalidixic acid (MIC, >32 μg/ml).

JIE273 was also highly resistant to amikacin, gentamicin, and tobramycin, and this was confirmed by E-test (AB Biodisk, Solna, Sweden) at >256, >1,024, and >256 μg/ml, respectively, suggesting the presence of a 16S rRNA methyltransferase (3). PCR screening of JIE273 for *armA* (8), *rmtA* (17), *rmtB* (19), *rmtC* (18), and *rmtD* (5) revealed *rmtC* only. This gene was originally discovered in *P. mirabilis* ARS68 in Japan in 2003 (16) and, to our knowledge, has not been reported elsewhere. The patient harboring JIE273 was born in and had recently returned to India but had no history of travel to Japan.

The PCR product obtained with primers ISEcpIR-F (5′-CAATGTGTGAGAAGCAGTCTAAA-3′) and rmtC-down (5′-GCGAGGTGTGTTAGAATTTGC-3′) contained a gene and flanking sequences identical to those of the original *rmtC* sequence with GenBank accession no. AB194779 (15). ISEcp*1* has been previously shown to promote both the expression and transposition of *rmtC* (15), and an additional PCR with IS*Ecp1*-5′ (6) and RMTC-R *rmtC* (18) confirmed a complete, uninterrupted copy of ISEcp*1* adjacent to *rmtC*, as in ARS68.

The *rmtC* gene was transferred from ARS68 to *Escherichia coli* by electroporation but not conjugation (16). Attempts to transfer *rmtC* from JIE273 to a rifampin-resistant variant of *E. coli* DH5α (DH5αRf) by a filter-based (14) or a broth-based (2) conjugation method with selection on amikacin (16 μg/ml) and rifampin (80 μg/ml) were unsuccessful. Repeated attempts to obtain amikacin-resistant (MIC, >16 μg/ml) transformants of *E. coli* DH5α by electroporation with DNA prepared from JIE273 by alkaline lysis (13) were also unsuccessful, despite successful transfer of control plasmids.

A "keyhole" effect in a double-disc synergy test (9) with 30-μg cefotaxime, ceftazidime, and aztreonam discs placed 15 mm from a 20/10-μg amoxicillin-clavulanate disc (Bio-Rad, Hercules, CA) suggested an extended-spectrum β-lactamase in JIE273. Known associations between 16S RNA methylases and extended-spectrum β-lactamase genes (3) include $bla_{\text{CTX-M}}$ and/or $bla_{\text{SHV}}$ with *armA* or *rmtB* (19). Neither the $bla_{\text{CTX-M}}$ (10), nor the $bla_{\text{SHV}}$ (12), nor the $bla_{\text{TEM}}$ (1) gene was amplified from JIE273, but a $bla_{\text{VEB}}$ (11) amplicon was obtained. A single nucleotide change from $bla_{\text{VEB-4}}$ (GenBank accession no. EF136375) predicts only a conservative substitution (Ile18Val) in the leader peptide. This β-lactamase was designated VEB-6 (http://www.lahey.org/Studies/), and we believe it is the first report of a $bla_{\text{VEB}}$-like gene in Australia.

The general problem of resistance to β-lactams, including carbapenems, is significantly exacerbated by aminoglycoside resistance, since they are often employed together in the treatment of the critically ill. In contrast to patterns observed elsewhere (4), amikacin susceptibility is currently preserved in gentamicin- and tobramycin-resistant isolates with transmissible metalloenzymes in Australia (7). However, the emergence of genes such as *rmtC* with a broad aminoglycoside resistance spectrum (including amikacin) will limit our future options for dealing with all β-lactamases.

**Nucleotide sequence accession numbers.** The nucleotide sequence of the *rmtC* gene has been submitted to GenBank under accession no. EU144360, and the $bla_{\text{VEB-6}}$ gene has been assigned accession no. EU259884.

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### REFERENCES

1. **Caniça, M. M., C. Y. Lu, R. Krishnamoorthy, and G. C. Paul.** 1997. Molecular diversity and evolution of $bla_{\text{TEM}}$ genes encoding β-lactamases resistant to clavulanic acid in clinical *E. coli*. J. Mol. Evol. **44:**57–65.
2. **Coque, T. M., A. Oliver, J. C. Perez-Diaz, F. Baquero, and R. Canton.** 2002. Genes encoding TEM-4, SHV-2, and CTX-M-10 extended-spectrum β-lactamases are carried by multiple *Klebsiella pneumoniae* clones in a single hospital (Madrid, 1989 to 2000). Antimicrob. Agents Chemother. **46:**500–510.
3. **Doi, Y., and Y. Arakawa.** 2007. 16S ribosomal RNA methylation: emerging resistance mechanism against aminoglycosides. Clin. Infect. Dis. **45:**88–94.
4. **Doi, Y., D. de Oliveira Garcia, J. Adams, and D. L. Paterson.** 2007. Coproduction of novel 16S rRNA methylase RmtD and metallo-β-lactamase SPM-1 in a panresistant *Pseudomonas aeruginosa* isolate from Brazil. Antimicrob. Agents Chemother. **51:**852–856.
5. **Doi, Y., A. C. Ghilardi, J. Adams, D. de Oliveira Garcia, and D. L. Paterson.** 2007. High prevalence of metallo-β-lactamase and 16S rRNA methylase coproduction among imipenem-resistant *Pseudomonas aeruginosa* isolates in Brazil. Antimicrob. Agents Chemother. **51:**3388–3390.
6. **Eckert, C., V. Gautier, and G. Arlet.** 2006. DNA sequence analysis of the genetic environment of various $bla_{\text{CTX-M}}$ genes. J. Antimicrob. Chemother. **57:**14–23.
7. **Espedido, B., J. Iredell, L. Thomas, and A. Zelynski.** 2005. Wide dissemination of a carbapenemase plasmid among gram-negative bacteria: implications of the variable phenotype. J. Clin. Microbiol. **43:**4918–4919.
8. **Galimand, M., P. Courvalin, and T. Lambert.** 2003. Plasmid-mediated high-level resistance to aminoglycosides in *Enterobacteriaceae* due to 16S rRNA methylation. Antimicrob. Agents Chemother. **47:**2565–2571.
9. **Jarlier, V., M. H. Nicolas, G. Fournier, and A. Philippon.** 1988. Extended broad-spectrum β-lactamases conferring transferable resistance to newer β-lactam agents in *Enterobacteriaceae*: hospital prevalence and susceptibility patterns. Rev. Infect. Dis. **10:**867–878.
10. **Mulvey, M. R., G. Soule, D. Boyd, W. Demczuk, and R. Ahmed.** 2003. Characterization of the first extended-spectrum β-lactamase-producing *Salmonella* isolate identified in Canada. J. Clin. Microbiol. **41:**460–462.
11. **Naas, T., P. Bogaerts, C. Bauraing, Y. Degheldre, Y. Glupczynski, and P. Nordmann.** 2006. Emergence of PER and VEB extended-spectrum β-lactamases in *Acinetobacter baumannii* in Belgium. J. Antimicrob. Chemother. **58:**178–182.
12. **Nüesch-Inderbinen, M. T., H. Hächler, and F. H. Kayser.** 1996. Detection of genes coding for extended-spectrum SHV β-lactamases in clinical isolates by a molecular genetic method, and comparison with the E test. Eur. J. Clin. Microbiol. Infect. Dis. **15:**398–402.
13. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory

manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

14. **Valenzuela, J. K., L. Thomas, S. R. Partridge, T. van der Reijden, L. Dijkshoorn, and J. Iredell.** 2007. Horizontal gene transfer within a polyclonal outbreak of carbapenem-resistant *Acinetobacter baumannii*. J. Clin. Microbiol. **45:**453–460.
15. **Wachino, J., K. Yamane, K. Kimura, N. Shibata, S. Suzuki, Y. Ike, and Y. Arakawa.** 2006. Mode of transposition and expression of 16S rRNA methyltransferase gene *rmtC* accompanied by IS*Ecp1*. Antimicrob. Agents Chemother. **50:**3212–3215.
16. **Wachino, J., K. Yamane, K. Shibayama, H. Kurokawa, N. Shibata, S. Suzuki, Y. Doi, K. Kimura, Y. Ike, and Y. Arakawa.** 2006. Novel plasmid-mediated 16S rRNA methylase, RmtC, found in a *Proteus mirabilis* isolate demonstrating extraordinary high-level resistance against various aminoglycosides. Antimicrob. Agents Chemother. **50:**178–184.
17. **Yamane, K., Y. Doi, K. Yokoyama, T. Yagi, H. Kurokawa, N. Shibata, K. Shibayama, H. Kato, and Y. Arakawa.** 2004. Genetic environments of the *rmtA* gene in *Pseudomonas aeruginosa* clinical isolates. Antimicrob. Agents Chemother. **48:**2069–2074.
18. **Yamane, K., J. Wachino, S. Suzuki, H. Kato, K. Shibayama, K. Kimura, K. Kumiko, I. Satoshi, Y. Ozawa, K. Toshifumi, and Y. Arakawa.** 2007. 16S rRNA methylase-producing, gram-negative pathogens, Japan. Emerg. Infect. Dis. **13:**642–646.
19. **Yan, J. J., J. J. Wu, W. C. Ko, S. H. Tsai, C. L. Chuang, H. M. Wu, Y. J. Lu, and J. D. Li.** 2004. Plasmid-mediated 16S rRNA methylases conferring high-level aminoglycoside resistance in *Escherichia coli* and *Klebsiella pneumoniae* isolates from two Taiwanese hospitals. J. Antimicrob. Chemother. **54:**1007–1012.

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