Antimicrobial Agents and Chemotherapy

The First Metallo-β-Lactamase Identified in Norway Is Associated with a TniC-Like Transposon in a *Pseudomonas aeruginosa* Isolate of Sequence Type 233 Imported from Ghana

Ørjan Samuelsen, Liselotte Buarø, Mark A. Toleman, Christian G. Giske, Nils O. Hermansen, Timothy R. Walsh and Arnfinn Sundsfjord

Antimicrob. Agents Chemother. **2009, 53(1):331. DOI: 10.1128/AAC.00785-08.**
Published Ahead of Print 17 November 2008.

ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Jan. 2009, p. 331–332 Vol. 53, No. 1
0066-4804/09/$08.00+0 doi:10.1128/AAC.00785-08

The First Metallo-β-Lactamase Identified in Norway Is Associated with a TniC-Like Transposon in a *Pseudomonas aeruginosa* Isolate of Sequence Type 233 Imported from Ghana▽

Metallo-β-lactamases (MBLs) are an emerging problem among various clinically important gram-negative bacilli, such as *Pseudomonas aeruginosa*, *Acinetobacter* spp., and *Enterobacteriaceae* (8).

Scandinavian countries are renowned for their low level of antibiotic resistance (1), and previous reports on the emergence of new resistance mechanisms have been associated with strain import, such as with the first Swedish MBL, derived from Greece (3).

As part of an ongoing national study of MBLs in clinical isolates of *P. aeruginosa*, the National Reference Centre received a carbapenem-resistant isolate (K34-7) from the Ullevål University Hospital in the autumn of 2006. The isolate was recovered from tracheal secretions upon admission of a patient who transferred to the hospital after prolonged hospitalization in Ghana. The isolate is therefore likely to have been imported to Norway from Ghana.

Susceptibility testing of the isolate using Etests (AB Biodisk, Solna, Sweden) showed that the isolate was susceptible only to colistin, intermediate to aztreonam, and resistant to other β-lactams (imipenem-meropenem MIC, >32 μg/ml), aminoglycosides, and fluoroquinolones according to EUCAST clinical breakpoints. The isolate had a positive MBL Etest ratio, and MBL production was confirmed by spectrophotometric analysis of imipenem hydrolysis by crude cell extracts and subsequent inhibition by EDTA (11). The sequence of the bla_{VIM-2} gene was confirmed by PCR using consensus primers for bla_{VIM}, and sequence analysis of the genetic context using oligonucleotides for the 5′ conserved sequence (5′CS), the 3′CS, bla_{VIM}, and *tniC* showed that the bla_{VIM-2} gene was located in an unusual class 1 integron flanked by the *tni* module similar to Tn*402* (7) and not the normal 3′CS end (fused *qacEΔ1-sul1*). PCR linking bla_{VIM-2} to *tniA*, *orf6*, and *tniB* and sequencing confirmed that the whole *tni* module was present. The gene cassette array of *aacA7-*bla_{VIM-2}*-dhfrB5-aacC-A5* is identical to other TniC-like transposons found in isolates from the United States (6), Russia (GenBank accession no. DQ522233), and Taiwan (12) and almost identical to a TniC-like transposon found in an Indian isolate (10). Multilocus sequence typing showed that K34-7 belonged to ST233, which is not part of any clonal complexes; however, Russian isolates with the same transposon belong to ST235, which is part of a clonal complex harboring MBL isolates from several countries in Europe (2, 4, 9). Further, pulsed-field gel electrophoresis (PFGE) analysis (SpeI digestion) and serotyping of K34-7 and isolates possessing TniC-like transposons from Russia (GenBank accession no. DQ522233) and Taiwan (12) showed that the isolates had different PFGE profiles and were of different serotypes (Russian isolate, O11; Taiwan isolate, O2; and K34-7, O6). Thus, the appearance of this TniC-like transposon in unrelated *P. aeruginosa* isolates suggests that the transposon is itself transferable and also responsible for the dissemination of bla_{VIM-2}. The chromosomal location of the TniC-like transposon was confirmed by hybridization of a radiolabeled bla_{VIM-2} probe to a chromosomal band larger than 1 megabase after I-Ceu-1 digestion of K34-7 genomic DNA and PFGE (5; data not shown). In conclusion, this study highlights the importance of TniC-like transposons in the global dissemination of bla_{VIM-2} and also the contribution of human population dynamics in spreading MBL genes.

(Part of this study was presented at the 17th European Congress of Clinical Microbiology and Infectious Diseases, Munich, Germany.)

Nucleotide sequence accession number. The nucleotide sequence determined in this study was deposited in the EMBL database under accession no. FM165436.

Ø.S. is supported by a grant from the Northern Norway Regional Health Authority Medical Research Program, and M.A.T. is funded by EU grant LSHM-CT-2005-018705.

The Taiwan isolate harboring the TniC-like transposon was kindly donated by Jing-Jou Yan.

REFERENCES

1. **European Antimicrobial Resistance Surveillance System (EARSS).** 2007. EARSS annual report 2006, p. 1–162. EARSS, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.
2. **Giske, C. G., B. Libisch, C. Colinon, E. Scoulica, L. Pagani, M. Fuzi, G. Kronvall, and G. M. Rossolini.** 2006. Establishing clonal relationships between VIM-1-like metallo-β-lactamase-producing *Pseudomonas aeruginosa* strains from four European countries by multilocus sequence typing. J. Clin. Microbiol. **44:**4309–4315.
3. **Giske, C. G., M. Rylander, and G. Kronvall.** 2003. VIM-4 in a carbapenem-resistant strain of *Pseudomonas aeruginosa* isolated in Sweden. Antimicrob. Agents Chemother. **47:**3034–3035.
4. **Libisch, B., J. Watine, B. Balogh, M. Gacs, M. Muzslay, G. Szabo, and M. Fuzi.** 2008. Molecular typing indicates an important role for two international clonal complexes in dissemination of VIM-producing *Pseudomonas aeruginosa* clinical isolates in Hungary. Res. Microbiol. **159:**162–168.
5. **Liu, S.-L., and K. E. Sanderson.** 1995. I-CeuI reveals conservation of the genome of independent strains of *Salmonella typhimurium*. J. Bacteriol. **177:**3355–3357.
6. **Lolans, K., A. M. Queenan, K. Bush, A. Sahud, and J. P. Quinn.** 2005. First nosocomial outbreak of *Pseudomonas aeruginosa* producing an integron-borne metallo-β-lactamase (VIM-2) in the United States. Antimicrob. Agents Chemother. **49:**3538–3540.
7. **Radstrom, P., O. Skold, G. Swedberg, J. Flensburg, P. H. Roy, and L. Sundstrom.** 1994. Transposon Tn*5090* of plasmid R751, which carries an integron, is related to Tn*7*, Mu, and the retroelements. J. Bacteriol. **176:** 3257–3268.
8. **Rossolini, G. M., and J. D. Docquier.** 2007. Class B β-lactamases, p. 115–144. *In* R. A. Bonomo and M. E. Tomasky (ed.), Enzyme-mediated resistance to antibiotics: mechanisms, dissemination, and prospects for inhibition. ASM Press, Washington, DC.
9. **Shevchenko, O., and M. Edelstein.** 2007. Epidemic population structure of MBL-producing *Pseudomonas aeruginosa* in Russia. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1499.
10. **Toleman, M. A., H. Vinodh, U. Sekar, V. Kamat, and T. R. Walsh.** 2007. bla_{VIM-2}-harboring integrons isolated in India, Russia, and the United States arise from an ancestral class 1 integron predating the formation of the 3′ conserved sequence. Antimicrob. Agents Chemother. **51:**2636–2638.
11. **Toleman, M. A., D. Biedenbach, D. M. Bennett, R. N. Jones, and T. R. Walsh.** 2005. Italian metallo-beta-lactamases: a national problem? Report

from the SENTRY Antimicrobial Surveillance Programme. J. Antimicrob. Chemother. **55:**61–70.
12. **Yan, J. J., P. R. Hsueh, J. J. Lu, F. Y. Chang, W. C. Ko, and J. J. Wu.** 2006. Characterization of acquired β-lactamases and their genetic support in multidrug-resistant *Pseudomonas aeruginosa* isolates in Taiwan: the prevalence of unusual integrons. J. Antimicrob. Chemother. **58:**530–536.

Ørjan Samuelsen*
Liselotte Buarø
Reference Centre for Detection of Antimicrobial Resistance
Department of Microbiology and Infection Control
University Hospital of North Norway
Tromsø, Norway

Mark A. Toleman
School of Medicine
Department of Medical Microbiology
Cardiff University
Cardiff, United Kingdom

Christian G. Giske
Department of Clinical Microbiology
Karolinska University Hospital
Stockholm, Sweden

Nils O. Hermansen
Department of Microbiology
Ullevaal University Hospital
Oslo, Norway

Timothy R. Walsh
School of Medicine
Department of Medical Microbiology
Cardiff University
Cardiff, United Kingdom

Arnfinn Sundsfjord
Department of Microbiology and Virology
IMB
Faculty of Medicine
University of Tromsø
Tromsø, Norway

*Phone: 47 776 27043
Fax: 47 776 27015
E-mail: orjan.samuelsen@unn.no

▽ Published ahead of print on 17 November 2008.